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Combined Efficacy of Organic Manures, Bio-Control Agents and Bio-Fertilizers in Improving Growth, Flowering and Quality Parameters of Gladiolus Cv. American Beauty

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A B S T R A C T

The present investigation was carried out to develop the organic farming practices of gladiolus cv. American Beauty utilizing bio-fertilizers and bio-control agents along with organic manures in 16 different combinations and the effects were compared with application of FYM only. The results indicated that growth and flowering as well as quality parameters were significantly influenced by integration of organic manures, bio-fertilizers and bio-control agents. The tallest plants (127.19 cm) and maximum number of leaves/ plant (14.04) were observed in the treatment consisting of Vermicompost (0.5 Kg/m^2) + Bio-inoculant treated corm + application of Bio-control agents (*Pseudomonas fluorescens* + *Trichoderma* @ 0.5Kg/m^2 each) + application of Nitrogenous Bio-fertilizers (*Azotobacter* + *Azospirilum* mixture @ 0.5Kg/m^2 each) + application of Phosphatic Bio-fertilizer (Phosphate solubilizing bacteria @ 0.5Kg/m^2) [T_{15}]. This treatment also recorded earliest spike emergence(60.48 DAP) and superiority in yield attributing characters like - spike length (93.06 cm), number of florets per spike(13.39), vase-life (6.90 days), weight of corm with scale leaf (72.34 g), weight of dehusked corms (70.57 g) and diameter of corms (69.16 mm). The quality parameters like chlorophyll, total phenol and protein content of leaves as well as anthocyanin content of florets were also influenced by combined use of organic manures and biological sources of nutrients. Hence, T_{15} may be considered as best supplementation of nutrients through organic approach and may be included in the package of practices for organic production of gladiolus in the Terai region of West Bengal.

Introduction

Gladiolus (*Gladiolus* sp.) is one of the most popular bulbous flowering ornamental plants with magnificent inflorescence. It is also known as the “Queen of bulbous flower crops” grown in many parts of the world. It is one of the principal flower crops of Terai region of West Bengal. The Terai region possesses unique climatological advantages, which is suitable for gladiolus cultivation for most part of the year (except in rainy season). Successful cultivation of gladiolus from this region often hinders due to some of the inherent problems of the agro-climatic condition as well as corm rot that threaten their economic value. Though the texture of the soil is suitable for production of bulbous plants, low pH of the soil and the associated problem of phosphorus fixation renders this nutrient element unavailable in most of the areas (Pati and Mukhopadhyay, 2008).

Gladiolus is a heavy feeder and it requires considerable amount of manures and fertilizers to produce quality spikes. The corm rot both in the field and in storage condition is another important problem of gladiolus cultivation. The pathogen may cause as much as 60–80% damage to gladiolus depending on varietal response (Devi *et al.*, 2017). However, the excessive use of chemical fertilizers/ fungicides to meet its nutritional requirement and/ or to control inherent diseases of corm rot may cause serious damage to the soil productivity and environment degradation (Sharma and Singhvi, 2017). Accumulation of toxic chemicals rendering the soil infertility, nutrient imbalance, ecosystem destruction, affects the yield and quality of the produce in the long run. In that condition, sustainable agricultural practices have become a very important consideration among the commercial growers. To overcome all these problems - a cheaper, better and safer way is necessary in order to improve the soil fertility status and sustainable production system with minimum Eco-hazards. All these criteria can be achieved through application of bio-fertilizers alternatively known as "microbial inoculants", are carrier-based preparations containing micro-organisms in sufficient numbers when applied as seeds treatment or soil application that accelerate certain microbial process that mobilized the available nutrient elements allowing to assimilate easily by plant also restricts the growth of disease producing organism helping plant growth and yield (Singh *et al.*, 2014). They are widely accepted as low-cost supplements to chemical fertilizers, save 25 % input (Thakur *et al.*, 2016) and have no deleterious effect either on soil health or environment.

Devi *et al.*, (2017) reported the use of chemical fungicides in regular practice in managing the diseases lead to a pollution problem, residual effects, toxicity, development of resistance in pathogen and imbalance in soil microbial associations. Application of bio-control agents is the alternative source to control/reduce the incidence of diseases like corm rot, wilt or yellow diseases and other harmful soil borne pathogen population and also exert the scope as a plant growth promoting rhizobacteria enhancing the plant growth, development and flowering in gladiolus (Sisodia and Singh, 2015). Several experiments were conducted to control this problem chemically and by use of bio-control agents but report on the use of a combination of bio-control agents and bio-fertilizers along with organic manures is very scanty. Keeping all these in view the present investigation was undertaken to find out the most effective combination in the package of practice for quality production of gladiolus through organic farming in the Terai region of West Bengal.

Materials and Methods

The experiment was conducted at the instructional farm of the Department of Floriculture, Medicinal and Aromatic plants, Faculty of Horticulture, Uttar Banga Krishi Viswavidyalaya, Pundibari, Cooch Behar during 2010- 2011 and 2011-2012.CoochBehar is geographically situated in the plains of the Terai zone of West Bengal especially in North Bengal at 26° N latitude and 89° E longitude and at an altitude of 43 m above the mean sea level. The climate of the region is humid sub-topical with hot summer and cold winter. The average annual rainfall varies from 2000-3000 mm, bulk of which being received during the monsoon (June to September) with a few pre-monsoon showers. The soil of the site of experiment was slightly acidic in nature (pH -5.38).

The design of experiment was Randomized Block Design (RBD) consisting of sixteen treatments replicated thrice. The treatments comprising of two types of organic manures [Farm yard manure (FYM) and vermicompost (VC)], two types of bio-control agents (*Pseudomonas fluorescens* and *Trichodermaviride*) and three types of bio-fertilizers (*Azotobacter*, *Azospirilum* and Phosphate solubilizing bacteria) with their combinations as given in Table 1.

The biological inoculants collected from the Department of Plant Pathology, Faculty of Agriculture, UBKV were used in five different heaps of the biological inoculants [*Trichoderma* (Strain UBT-18), *Pseudomonas fluorescens* (Strain VPF-1), *Azotobacter* (Strain UBAZ-1), *Azospirillum* (Strain UBAS-1)and Phosphate solubilizing bacteria (Strain UBPS-9)] with vermicompost @ 10g/Kg were prepared seven days before planting. The microbial resource enriched vermicompost was then applied separately from each heap as per the treatment during the planting of corms at the rate of 0.5 kg mixture/m^2. Uniform size of corms of gladiolus cv. American Beautywere treated with Carbendazim @ 1g/lit of water for ½ an hour for the treatments (T_2, T_4, T_6, T_8, T_{10}, T_{12}, T_{14} and T_{16}) and the remaining other corms (for treatments T_1, T_3, T_5, T_7, T_9, T_{11}, T_{13} and T_{15}) were treated with slurry of bio-inoculants prepared with CMC (carboxymethyl cellulose sodium salt) dipped for 5-10 minutes prior to planting. Treated corms were planted inraised beds at the spacing of 30cm × 30 cm (9 corms/ bed). All the cultural practices were kept uniform for all the treatments and standard practices were adopted. Observations on growth, flowering and corm production attributes were recorded from five randomly selected plants of each replication. Chlorophyll content of the leaves were done using chlorophyll meter - SPAD 502. The other parameters like protein content was estimated following Lowry*et al.*,(1951), leaf total phenol content following Malick and Singh (1980) and anthocyanin pigment concentration of floret following Cordenunsi *et al.*, (2003). Data of both the years were pooled and subjected to analysis of variance to determine the differences among group means was done following Ronald Fisher's statistical hypothesis testing technique through MSTATC (Mathematic and Statistic Compiler) software. The critical difference between the treatments was also determined at 5% level.

Results and Discussion

The pooled data (Table 2 and 3) reflected that most of the attributes were significantly affected by the combined treatment of organic manures, bio-control agents and bio-fertilizers and the effects were compared with application of only FYM @ 5 Kg/m^2+ Chemical corm treatment (T_2, control) in the field. T_{15} produced the tallest plants (74.46 cm) at 30 days after planting (DAP) and at 60 DAP (98.79 cm) which was statistically at par with T_{16}, T_8, T_7, T_{14}, T_{13} (at 30 DAP) andT_{16}, T_8, T_7 (at 60 DAP). Whereas at 90 DAP, T_{15} produced significantly tallest plants (127.19 cm) over the rest of treatments. The same treatment (T_{15}) also induced maximum number of leaves/ plant at 30 DAP (12.92), 60 DAP (13.87) and at 90 DAP (14.04).Whereas, the lowest plant height (62.38 cm, 76.46 cm and 97.88 cm) and the lowest number of leaves/ plant (9.00, 9.25 and9.38) were recorded inT_2, respectively at 30, 60 and 90 DAP (Table 2). The favourable effect of T_{15} to produce more vegetative growth might be due to the enhancement in the ability of the plants through application of those biological resources (corm treatment as well as soil application) through better uptake of nutrient elements, solubilisation and mobilisation of insoluble form of phosphorous in the soil, better photosynthetic ability, enhanced source-sink relationship which facilitated the physiological and biochemical activities at a higher magnitude (Kumar *et al.,* 2011) and antagonistic of bio-control agents to many phytopathogenic fungi against disease incidence as well as plant growth promoting rhizobacteria (Sisodia and Singh, 2015). Besides, the applied organic manures vermicompost possibly supplied the macro and micro (Zn, Cu, Fe, and Mn) nutrients, enzymes and growth promoting substances (Kumar *et al.,* 2011) lead to enhanced vegetative growth. Improved physiological and biological activities enhanced biological efficiency of the plant enabling synthesis of maximum metabolites and photosynthates ultimately encouraging quick growth in the form of plant height and leaf production. The effect of microbial resources and organic manure on improved vegetative growth of gladiolus was also reported by Sathyanarayana *et al.,* (2018) at100% RDF + FYM @ 7.5 t/ha + Azotobacter + PSB + KMB + 1% foliar spray of Nauroji Novel Organic Liquid Fertilizer application and Pandey *et al.,* (2013) through corm inoculation as well as soil application biocontrol agents + vermicompost.

The earliest (60.48 DAP) spike emergence was noticed in T_{15} which was at par with T_{16}, $T_8$and maximum delay (64.81 DAP) was noticed in T_1 (Table 2). Earlier completion of better vegetative growth lead to early flowering is due to improved physiological and biochemical activities as well as rate of photosynthesis in T_{15}. PSB induced better uptake of phosphorus as well as micro-nutrient like Zn, a precursor of auxin biosynthesis, might lead to improved vegetative growth, dry matter accumulation, better photosynthetic ability and supply of photosynthates and their partitioning towards the initiation of floral primordia (Dubey *et al.,* 2010). The use of organic manure supplemented biological resources for earlier completion of better vegetative growth as well as early initiation of flower bud was also noticed by Kuotsu*et al.,* (2018) in gladiolus; Srivastava *et al.,* (2014) in tuberose and Kumar *et al.,* (2017) in tomato. Similar results in early flowering and floret opening in gladiolus (Sisodia and Singh, 2015) through application of *Trichoderma* along with vermicompost probable reason may be effective control of diseases and its opportunistic role to enhance plant growth. Similarly, T_{15} was found as the most improved performer with regard to longest (93.06 cm) spike length, maximum number (13.39) of florets per spike and maximum (6.90 days) of vase-life which were statistically at par with T_{16}, $T_8$and T_7 (Table 2). Whereas, T_1 recorded the lowest spike length (69.78 cm), number of florets per spike (8.28) and vase-life (5.06) followed by T_2. The quality parameters like spike length, number of florets/ spike and post-harvest life of cut spikes were improved considerably in T_{15} might be due to their effect in facilitating better partitioning of nutrients resulting better nutrition followed by growth promotion along with proper root function favoured better reproductive growth which was reflected through the parameters. The microbial resources and organic manure mediated improvement in spikes quality parameters was also noticed by Sathyanarayana *et al.,* (2017), Pansuriya *et al.,* (2018) and Ali *et al.,* (2014) in gladiolus. Beneficial effect of bio-control agent and vermicompost in improving the quality parameters of spikes in gladiolus has been documented by Pandey *et al.,* (2013). The improved stored food reserves within the spike as a result of better nutrition during the vegetative and reproductive phases as well as formation of cytokinin like plant growth substances along with auxin and GA_3influenced higher spike length and better post-harvest life of cut spikes as the anti-senescence property of cytokinin is well versed (Srivastava *et al.,* 2014) which was also supplemented through increased carbohydrate reserves within the cut spikes.

The findings of the present experiment in respect of vase life of spikes are also in close proximity of the findings of Pansuriya *et al.*, (2018) and Sathyanarayana *et al.*, (2018) in gladiolus.

Highly significant difference was observed among different treatments in corm quality of gladiolus cv. American Beauty (Table 3). T_{15} produced the maximum weight of corm with scale leaf (72.34 g) and weight of dehusked corms (70.57 g), which were statistically at par with T_{16}, T_8, T_7, T_{14} and T_{13}.Whereas $T_2$was found with minimum weight of corm with scale leaf (52.21 g) as well as dehusked corms (50.41 g). Similarly, the maximum diameter of corms (69.16 mm) was recorded in T_{15}, which was statistically at par with T_{16}, T_8 and the minimum diameter of corms (61.23 mm) was noticed in T_2.

The improved weight and sizes of corms in T_{15} might be due to the integrated effect exerted by the nitrogen fixing microbes to fix higher amount of atmospheric nitrogen at the root zone and to make it available for utilization of the crop; phosphorous solubilizing bacteria to release the phosphorous that helps in better root growth thereby translocation efficiency of phosphorous and other micronutrients resulting increased biosynthesis of chlorophyll to facilitate photosynthesis thereby production and distribution of photosynthates for all round development of the crops. Besides, the bio-control agent that imparted disease resistance to the crop and helped in biosynthesis of growth promoting substances (Sisodia and Singh, 2015) as well as made the rhizosphere healthy for successful growth and the organic manure like vermicompost which were considered as a supplier of nutrient, slowly, throughout the crop growth, development, flowering and post-harvest corm formation period on a continual basis, responsible for supply of macro and micro nutrient elements essential to gladiolus plant.

As a result, plants became able to synthesise more assimilates and after harvesting of flowers which were channelized to the storage organ leading to formation of better quality corms. Similar kind of results was also noticed by Sathyanarayana *et al.,* (2018) in gladiolus and Naznin *et al.,* (2015) in tuberose.

It is also revealed from the data presented in Table 3 that the highest chlorophyll content (67.07 SPAD) of leaves, leaf protein content (7.20 mg/g of fresh weight)and floret anthocyanin content (214.17 mg/ 100g of fresh weight) were obtained from T_{15} which was statistically at par withT_{16}, T_8, T_3, T_7, T_{14}, T_{13}, T_5, T_6 (in case of leaf chlorophyll content), T_{16}, T_8, T_7, T_4 (in case of leaf protein content) and T_{16}, T_7, T_8, T_{14}, T_{13} (in case of floret anthocyanin content). The minimum leaf chlorophyll content (61.32 SPAD), leaf protein content (3.96mg/g of fresh weight) and floret anthocyanin content (176.67 mg/ 100g of fresh weight) were found in T_2. Also, T_{15} recorded with highly significant Phenol (0.96mg/g fresh weight) content of leaves over the rest of treatments and lowest phenol content (0.42mg/g fresh weight) was observed in T_2.

Nitrogen, the essential part of nucleic acid, was supplied continuously at a steady rate by the manures and microbes together enhancing more vegetative growth.

Table.1 Treatments combination details

Notations	Treatment details
T_1	FYM (5 Kg/m^2) + Bio-inoculant treated corm (BTC)
T_2	FYM (5 Kg/m^2) + Chemical treated corm (CTC)
T_3	FYM (5 Kg/m^2) + BTC + Bio-control agent (a mixture of *Pseudomonas fluorescens* + *Trichoderma* @ 0.5 Kg/m^2 each)
T_4	FYM (5 Kg/m^2) + CTC + Bio-control agent
T_5	FYM (5 Kg/m^2) + BTC + Bio-control agent + Nitrogenous Bio-fertilizer (a mixture of *Azotobacter* + *Azospirilum*@ 0.5 Kg/m^2 each)
T_6	FYM (5 Kg/m^2) + CTC + Bio-control agent + Nitrogenous Bio-fertilizer
T_7	FYM (5 Kg/m^2) + BTC + Bio-control agent + Nitrogenous Bio-fertilizer + Phosphatic Bio-fertilizer (Phosphate solubilizing bacteria @ 0.5 Kg/m^2 each)
T_8	FYM (5 Kg/m^2) + CTC + Bio-control agent + Nitrogenous Bio-fertilizer + Phosphatic Bio-fertilizer
T_9	VC (0.5 Kg/m^2) + BTC
T_{10}	VC (0.5 Kg/m^2) + CTC
T_{11}	VC (0.5 Kg/m^2) + BTC + Bio-control agent
T_{12}	VC (0.5 Kg/m^2) + CTC + Bio-control agent
T_{13}	VC (0.5 Kg/m^2) + BTC + Bio-control agent + Nitrogenous Bio-fertilizer
T_{14}	VC (0.5 Kg/m^2) + CTC + Bio-control agent + Nitrogenous Bio-fertilizer
T_{15}	VC (0.5 Kg/m^2) + BTC + Bio-control agent + Nitrogenous Bio-fertilizer + Phosphatic Bio-fertilizer
T_{16}	VC (0.5 Kg/m^2) + CTC + Bio-control agent + Nitrogenous Bio-fertilizer + Phosphatic Bio-fertilizer

Table.2 Effect of organic manures, bio-control agents and bio-fertilizers on plant growth and floral characteristic of gladiolus cv. American Beauty

Treatment (T)	Plant height			No. of leaves/plant			FBI (days)	Spike length (cm)	No. of florets/ spike	Vase-life of spike (days)
	30 DAP	60 DAP	90 DAP	30 DAP	60 DAP	90 DAP				
T_1	67.96	84.21	99.88	10.00	10.33	10.54	64.81	69.78	8.28	5.065
T_2	62.38	76.46	97.88	9.00	9.25	9.38	63.53	71.56	8.39	5.112
T_3	69.29	87.75	105.17	11.21	11.96	11.96	62.72	76.94	9.17	5.337
T_4	69.35	88.92	105.50	11.04	12.13	12.21	63.03	80.56	9.72	5.347
T_5	70.33	89.42	112.13	11.25	11.46	11.54	63.50	82.28	11.39	6.445
T_6	70.63	93.96	110.00	11.50	11.79	12.17	62.42	84.28	12.00	6.550
T_7	73.21	97.04	116.00	12.63	12.25	12.29	63.14	88.11	12.11	6.767
T_8	73.50	95.96	115.21	12.17	12.08	12.33	61.87	89.06	12.44	6.610
T_9	66.13	86.42	100.19	10.04	10.58	10.75	64.59	74.28	8.83	5.435
T_{10}	68.67	86.58	104.63	10.25	10.96	11.08	64.67	73.95	9.06	5.120
T_{11}	69.08	88.29	106.92	11.29	11.92	12.00	64.03	81.33	10.17	5.585
T_{12}	69.33	88.29	107.08	10.42	11.00	11.21	64.70	80.67	10.00	5.600
T_{13}	71.17	94.38	113.96	11.37	12.04	12.04	63.59	84.34	11.95	6.545
T_{14}	71.67	94.21	113.81	11.54	11.33	11.38	64.42	87.67	10.22	6.585
T_{15}	74.46	98.79	127.19	12.92	13.87	14.04	60.48	93.06	13.39	6.900
T_{16}	73.79	98.46	116.21	12.79	13.58	13.79	61.70	90.44	12.67	6.800
SEm(±)	1.260	1.423	1.775	0.703	0.588	0.585	0.592	2.035	0.491	0.308
CD (P=0.05)	3.564	4.025	5.128	1.989	1.663	1.655	1.675	5.757	1.389	0.871

Table.3 Effect of organic manures, bio-control agents and bio-fertilizers on corm and biochemical quality parameters of gladiolus cv. American Beauty

Treatment (T)	Weight of corm with scale leaf (gm)	Weight of corm after dehusked (gm)	Diameter of corm (mm)	Chlorophyll content (SPAD)	Protein Content (mg/g of fresh wt.)	Phenol (mg/g fresh wt.)	Anthocyanin (mg/100g)
T_1	53.81	52.73	61.91	61.34	4.79	0.47	180.80
T_2	52.21	50.41	61.23	61.32	3.96	0.42	176.67
T_3	65.91	61.36	64.79	66.03	6.37	0.56	189.07
T_4	62.66	62.16	63.21	64.18	6.44	0.62	186.90
T_5	63.48	59.78	65.83	65.32	6.35	0.55	193.92
T_6	62.33	59.65	64.66	64.99	6.28	0.61	194.87
T_7	68.83	66.85	66.08	66.03	6.69	0.80	206.28
T_8	69.40	67.53	66.62	66.27	6.74	0.83	203.42
T_9	58.50	55.60	63.00	62.52	4.28	0.50	192.57
T_{10}	58.23	57.08	62.54	63.18	4.98	0.53	177.21
T_{11}	64.34	63.82	64.49	62.29	6.29	0.55	198.53
T_{12}	65.72	63.24	64.47	63.84	6.23	0.66	198.67
T_{13}	67.87	65.77	65.79	65.96	6.26	0.58	199.97
T_{14}	68.28	66.33	63.37	65.99	5.81	0.72	203.05
T_{15}	72.34	70.57	69.16	67.07	7.20	0.96	214.17
T_{16}	72.25	69.87	68.30	66.49	6.86	0.83	206.54
SEm(±)	2.012	2.056	0.935	0.887	0.289	0.010	5.249
CD (P=0.05)	5.811	5.938	2.645	2.509	0.818	0.028	14.849

Highly significant total chlorophyll content as well as higher accumulation of various metabolites (reducing sugar, total phenol and amino nitrogen) might have resulted from enhanced plant growth and biomass production (Kohler *et al.*, 2007). Use of PSB on the other hand rendered the phosphorus to be available to the plants leading to enhanced chlorophyll biosynthesis thereby improving many physiological processes like cell division, carbohydrate, fat and protein metabolism (Ali *et al.*, 2014). Similar kind of results were noticed by Khalid *et al.*, (2017) with the highest chlorophyll content upon inoculation with *A. chroococcum*, *B. megaterium* and *B. mucilaginous* bacterial strains and highest total phenol and flavonoid content upon inoculation with mycorrhizal fungi (*Glomus fasciculatum*) in spinach. Increased protein synthesis in T_{15} may be attributed due to increased availability and uptake of nitrogen, as nitrogen is the most important element in protein synthesis and its increase in optimum conditions increases the amount of protein (Rahmani *et al.*, 2008). Anthocyanin is the major contributor of pigmentation in gladiolus flowers (Takemura *et al.*, 2008). Anthocyanin biosynthesis took place from anthocyanidin by addition of sugars. Plant Growth Promoting Rhizobacteria influences the anthocyanin formation in plants (Rodriguez *et al.*, 2014). Application of *Azotobacter* and PSB enhanced the anthocyanin formation (Selvarathi *et al.*, 2010) in tomato.

The present investigation revealed that the treatment "T_{15}"comprisedof Vermicompost (0.5 Kg/m^2) + Bio-inoculant treated corm + Bio-control agent (a mixture of *Pseudomonas fluorescens* + *Trichoderma* @ 0.5 Kg/m^2 each)+ Nitrogenous Bio-fertilizer(a mixture of *Azotobacter* + *Azospirilum*@ 0.5 Kg/m^2 each)+ Phosphatic Bio-fertilizer(Phosphate solubilizing bacteria @ 0.5 Kg/m^2) showed significant increase in the growth, flowering and quality parameters of gladiolus cv. American Beauty as compared to other treatments. Hence, T_{15} may be considered as best organically nutrient supplementation in the package of practices for organic production of gladiolus in the Terai region of West Bengal.

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