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Ameliorate the pre-flowering growth of *Gladiolus grandiflorus* L. Red majesty with composted coconut coir as organic amendment

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Abstract
Production of cut flowers as well as plants depend on dynamic pre-flowering (vegetative) growth. Pre-flowering growth depends on the quantity and availability of macro- and micronutrients in the soil. Organic fertilizers and bio-fertilizers can renovate nutritionally essential elements from unavailable to available form through biological procedures leading to crop yields. In present research, composted coconut coir as organic fertilizers in combinations with Trichoderma harzianum (as bio-fertilizer) and Ferula asafoetida (as biocontrol agent) used as treatments. Experiment was conducted in the Department of Botany, Jinnah University for Women in complete randomized design as an experimental unit with four replicates. Data were collected after 30 days. Most of the selected treatments promote the growth of Gladiolus plants significantly. The results showed increase in root length, shoot length, leaf area, fresh and dry weight of root and shoot of experimental plants. All treatments also increased the biochemical contents of experimental plants. The results of the given research proved that the organic fertilizers with live formulates of microorganisms (useful bacteria and fungi) restoring the natural fertility and soil diseases and therefore stimulate plant growth.

Keywords: Cut flowers, pre-flowering growth, soil fertility, compost, coconut coir

1. Introduction
Gladiolus grandiflorus L. belongs to Iridaceae family. Red majesty is an important ornamental and commercial flower known as queen of the bulbous plants [1]. It is the second most popular cut flower in Pakistan after Rose species that is grown for both as potted and aesthetic cut flower in the country. Commercial production of gladiolus can generate not only good income but can also fetch foreign exchange through their export [2]. The propagating material of gladiolus is called "corm" which is a food-storing underground stem [3]. Gladiolus requires well drained soil for achieving healthy plants. They also need open position for getting more sunlight, ample water with heavy soaking weekly.
Research has shown that organically produced food crops are healthier for consumption and safer for the environment. Soil fertility can be presumably enhanced by organic and inorganic fertilizers application [4]. The use of organic fertilizers will provide an environmentally friendly, naturally sustainable, safe and affordable means for maintaining soil fertility and increasing crop production. Composts are known to suppress plant diseases through a combination of physiochemical and biological characteristics [5]. Incorporation of composted coconut coir improved nutrient availability to plants in turn improving growth and quality. It also enhances soil fertility, plant nutrient status, saves cost of secondary and micro-nutrients required for obtaining good yields and leads to less environmental pollution [6]. Gladiolus producers all over the world suffer heavy losses due to a large number of fungal diseases. There is a need for some eco-friendly biocontrol agents that may help to resolve some of these problems [7]. *Trichoderma* is widely used as biocontrol agent against phyto-pathogenic fungi, and as a bio-fertilizer because of its ability to establish mycorrhiza like association with plants [8]. Soil amendment with asafoetida in general showed suppressive effect on soil borne pathogens and have a positive effect on growth of plants [9].

2. Materials and methods
2.1 Experimental Site
The research work was carried out at the net house of department of Botany, Jinnah University for Women, Karachi.

2.2 Plant material
In this experiment Gladiolus (Gladiolus grandiflorus L.) cv. "Red Majesty" was used as experimental plant.

2.3 Fertilizers and Fungicide used in present experimental work
Coconut coir used as composted agricultural wastes and *Trichoderma harzianum* as bio fertilizer & bio fungicide. Asafoetida as organic bio control agent.

2.4 Composting
Coconut coir was composted aerobically with Trichoderma harzianum for 30 days.

2.5 Treatments used in experimental work

Table 1: List of Treatments.

S. no.	Treatments
1	Control
2	Coconut coir 10 ton/ha
3	Coconut coir 20 ton/ha
4.	*Trichoderma harzianum* @ 8×10^9
5.	Asafoetida @ 200 ppm/ml
6.	Coir 10 ton/ha + Trichoderma 8×10^9
7.	Coir 20 ton/ha + Trichoderma 8×10^9
8.	Coir 10 ton/ha + Asafoetida @ 200 ppm/ml
9.	Coir 20 ton/ha + Asafoetida @ 200 ppm/ml

2.6 Experimental Design and layout of the experiment
The experiment was laid out in Randomized Complete Block Design with four replications. Nine treatments including a control (soil only) were assigned randomly to the pots containing 1.5kg soil.

2.7 Irrigation
Irrigation was applied by observing the soil moisture condition.

2.8 Harvesting
After 90 days plants from each treatment were plugged out. After harvesting root and shoot lengths, root and shoot fresh and dry weights, leaf area (cm^3) and biochemical parameters including total chlorophyll by using 80% acetone [10], carbohydrate by using Anthrone methods [11], % protein contents by Bradford method [12] were recorded in this study.

3. Results
All treatments increased root lengths of experimental plants which shown in figure 1.Coir 20 tons/ha with *Trichoderma harzianum* @ 8×10^9 cfu and Asafoetida @200ppm/ml significantly promoted root lengths of Gladiolus plants(fig.1), while all treatments significantly increased shoot lengths of experimental plants after 30 days of growth as compared with control(fig.1). Maximum shoot lengths observed with the treatment of coir 20 tons/ha in combination with asafoetida @200ppm/ml (fig.1).

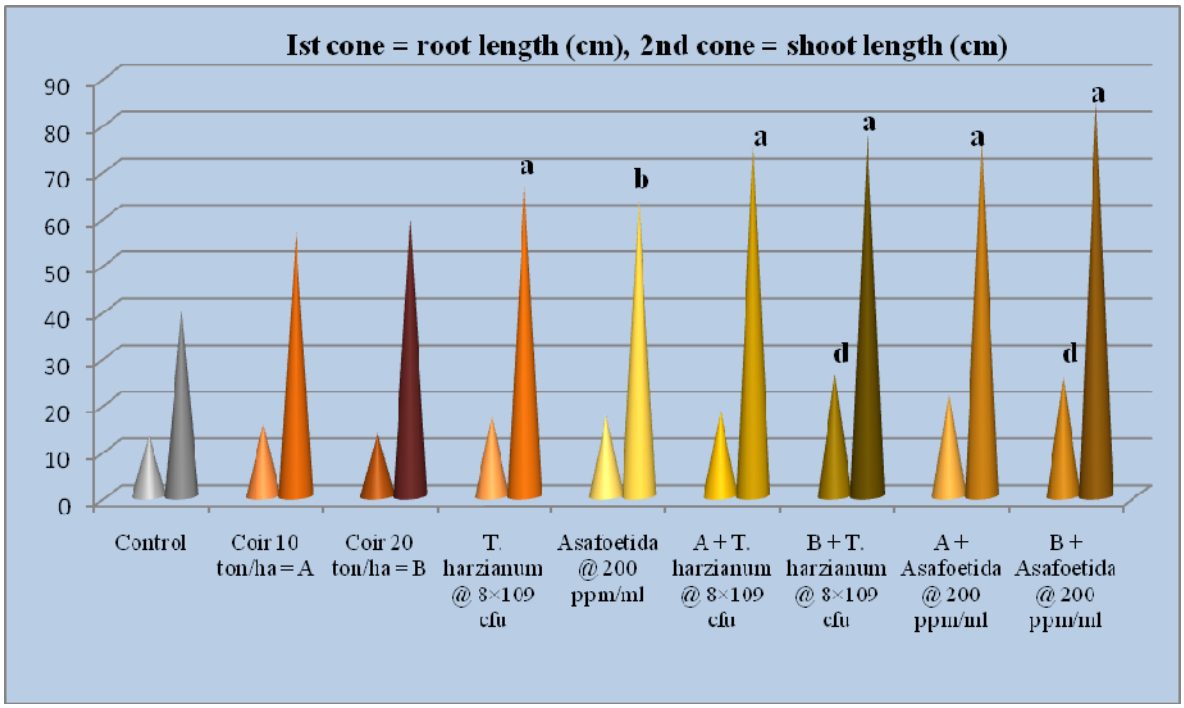

Fig 1: Effect of composted coconut coir on root & shoot lengths of gladiolus plants. Columns bearing superscript are statistically significant ($p<0.05$ LSD) with respective control. a = $p<0.0001$, b = $p<0.001$, c = $p<0.01$ andd = $p<0.05$.

Most of the treatments enhanced fresh weight of roots and shoots significantly as compared with control (fig.2). Most significant result observed in both root and shoot fresh weights with the treatments of *T. harzianum* @ 8×10^9 cfu alone and coir 20tons/ha in combination with asafoetida @ 200ppm/ml (fig.2).

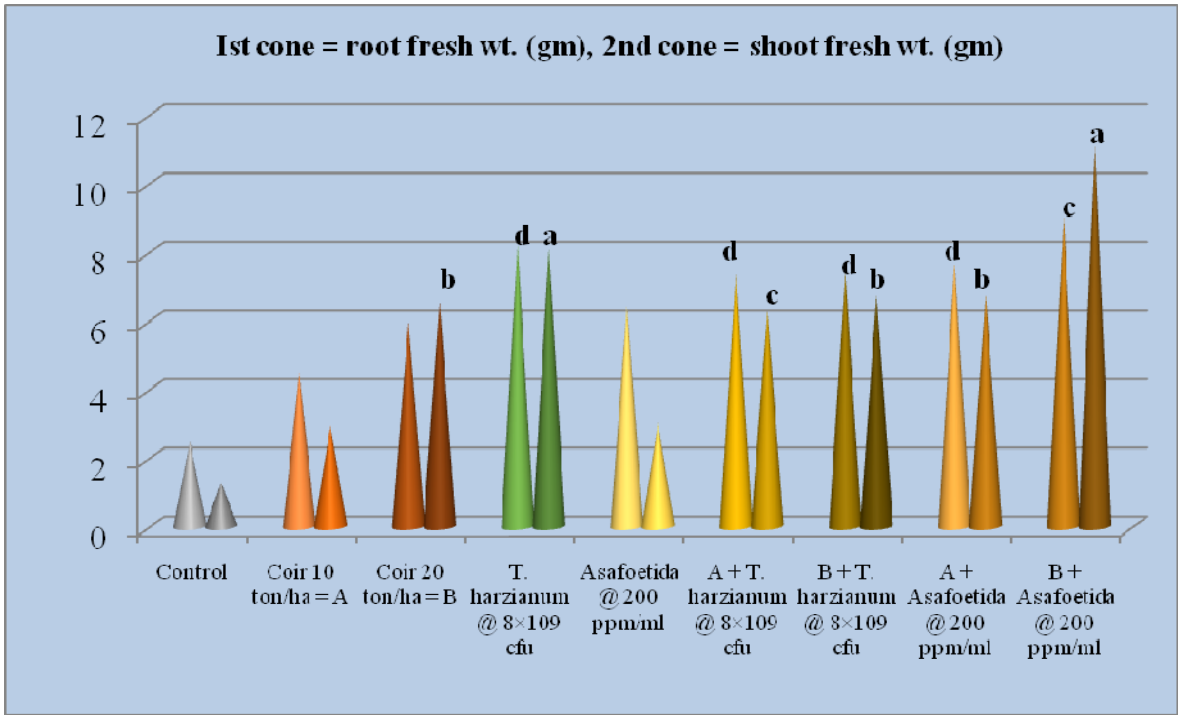

Fig 2: Effect of composted coconut coir on root & shoot fresh weights of gladiolus plants. Column bearing superscript are statistically significant (p<0.05 LSD) with respective control. a = p<0.0001, b = p<0.001, c = p<0.01 a =p<0.05.

Root dry weights of gladiolus plants also promoted with all treatments as compared with control after 30 days of growth. Maximum increased was observed with the treatments of *T. harzianum* @ 8×10^9 cfu alone up to 227% and with coir 10tons/ha in combination with asafoetida @200ppm/ml (fig.3). Most significant results of shoot dry weights of experimental plants were obtained when plants were treated with coir 20tons/ha alone and coir 20tons/ha in combination with asafoetida @ 200ppm/ml (fig.3).

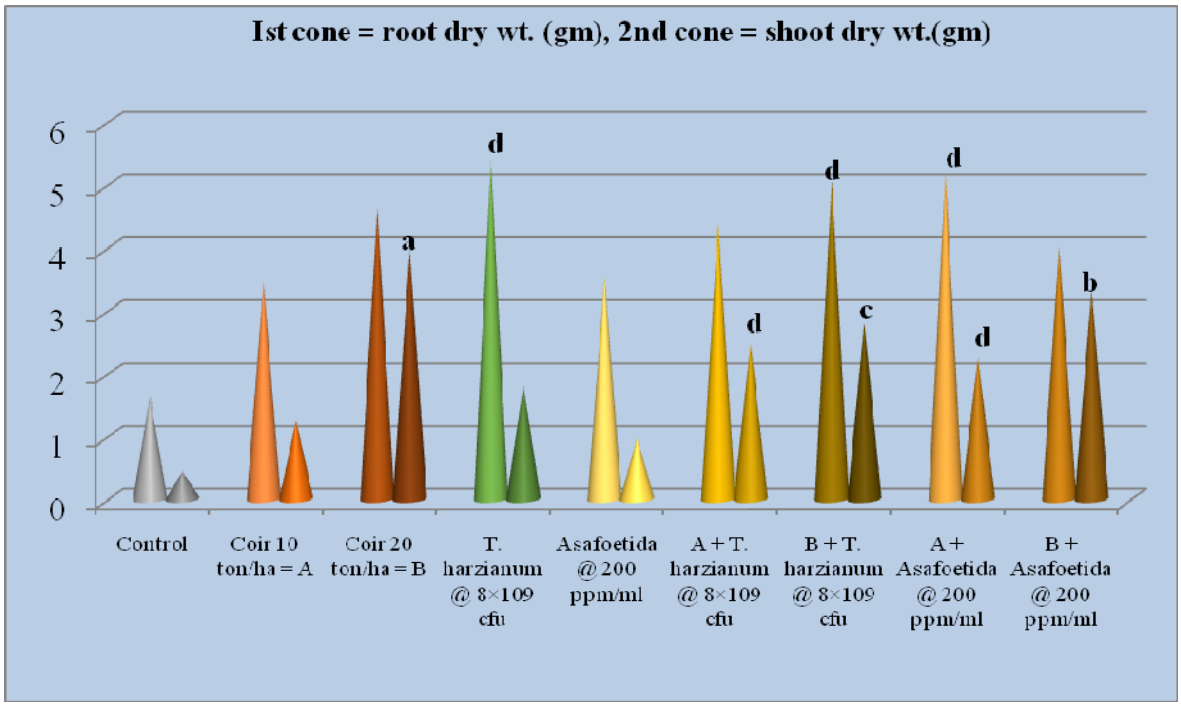

Fig 3: Effect of composted coconut coir on root & shoot dry weights of gladiolus plants. Column bearing superscript are statistically significant (p<0.05 LSD) with respective control. a = p<0.0001, b = p<0.001, c = p<0.01 and d = p<0.05.

Number of leaves as shown in fig.4 significantly increased only with the treatment of coir 20tons/ha in combination with asafoetida 200ppm/ml. All experimental treatments promoted leaf area of gladiolus plants significantly as compared with control after 30 days of growth as shown in fig.5. Maximum increased in leaf area was observed with the treatment of coir 20tons/ha in combination with *T. harzianum* @ 8×10^9 cfu and with asafoetida @ 200ppm /ml in experimental plants (fig.5).

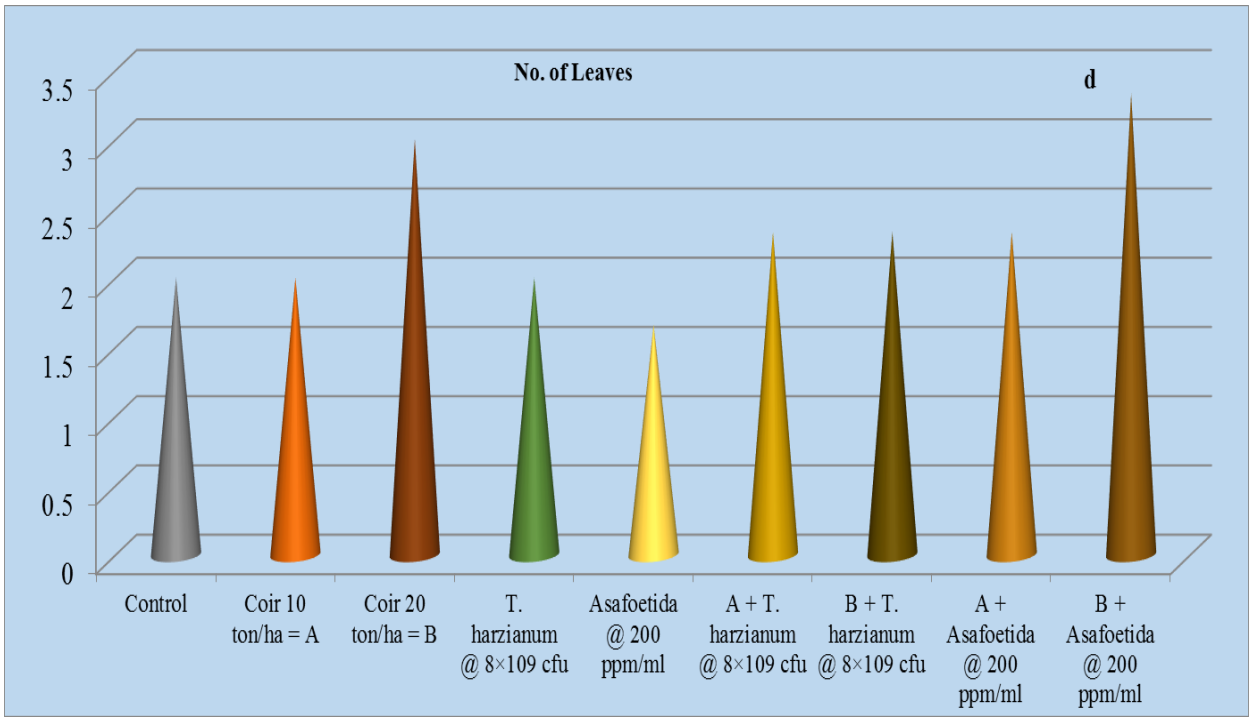

Fig 4: Effect of composted coconut coir on number of leaves of gladiolus plants. Column bearing superscript are statistically significant (p<0.05 LSD) with respective control. a = p<0.0001, b = p<0.001, c = p<0.01 and d = p<0.05.

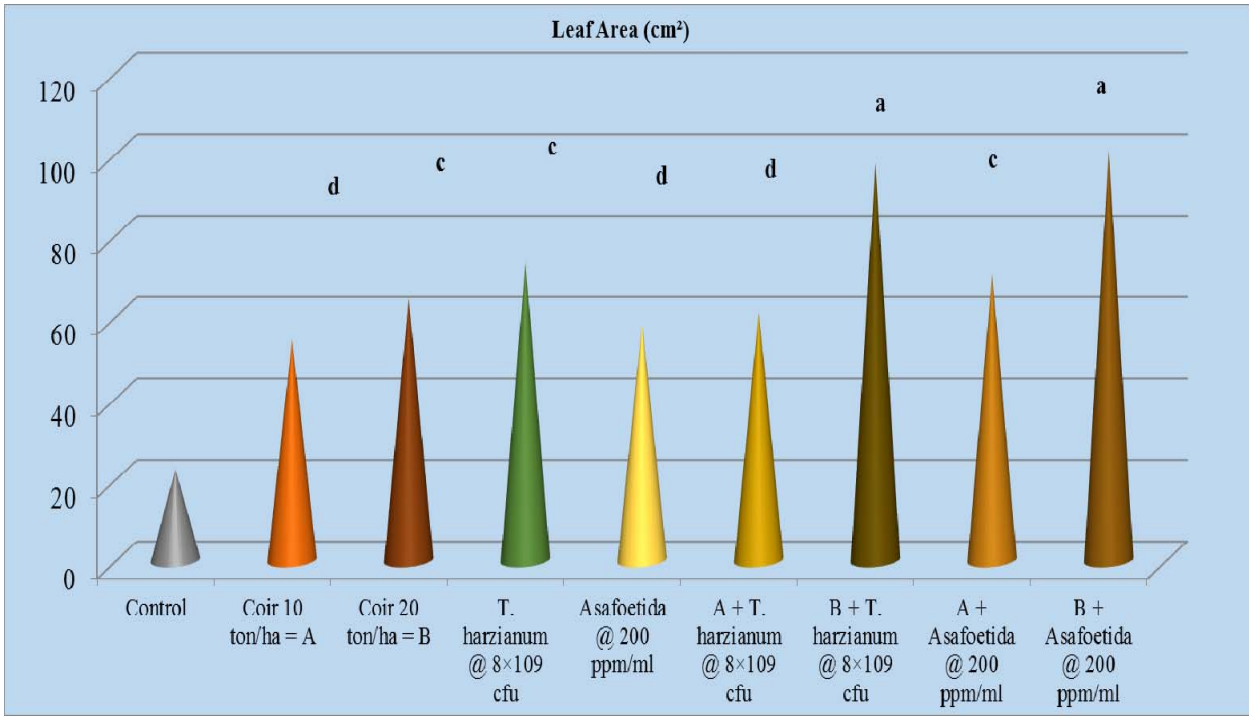

Fig 5: Effect of composted coconut coir on leaf area of gladiolus plants. Columns bearing superscript are statistically significant (p<0.05 LSD) with respective control. **a** = p<0.0001, **b** = p<0.001, **c** = p<0.01 and **d** = p<0.05.

Biochemical parameters including total chlorophyll (mg/g fresh wt.), % protein and % carbohydrate were also promoted with all given treatments of gladiolus plants as compared with control after 30 days of growth (tab.,2). Only treatment with coir 20tons/ha in combination with *T. harzianum* @ 8×10^9 cfu significantly enhanced the total chlorophyll content of experimental plants. Most of the treatments significantly enhanced % protein content of plants. Both *T. harzianum* @ 8×10^9 cfu and Asafoetida @ 200ppm/ml alone promoted maximum % protein in experimental plants (tab., 2). Coir 10 tons/ha alone and in combination with *T. harzianum* @ 8×10^9 cfu significantly enhanced % carbohydrate content of gladiolus plants as compared with control (tab., 2).

Table 2: Effect of composted coconut coir on Total chlorophyll, % Protein and %Carbohydrate contents of gladiolus plant.

Treatments	Total Chl. (mg/gm fresh wt.)	Protein (%)	Carbohydrate (%)
Control	Mean ± Std. Dev.	Mean ± Std. Dev.	Mean ± Std. Dev.
	0.73 ± 0.16	23.66 ± 5.91	26.92 ± 21.68
	0	0	0
Coir 10 ton/ha = A	0.97 ± 0.23	32.35 ± 4.11	55.64 ± 25.57 [c]
	(+ 32.87)	(+ 36.72)	(+ 106.68)
Coir 20 ton/ha = B	0.84 ± 0.30	37.33 ± 6.98	31.25 ± 6.91
	(+ 15.06)	(+57.76)	(+ 16.08)
T. harzianum @ 8×10^9 cfu	1.21 ± 0.12	82.06 ± 20.93 [a]	47.72 ± 10.92
	(+ 65.75)	(+ 246.83)	(+ 77.26)
Aesofoetida @ 200 ppm/ml	0.79 ± 0.10	89.44 ± 23.71 [a]	39.91 ± 7.78
	(+ 8.21)	(+ 278.02)	(+ 48.25)
A + T. harzianum @ 8×10^9 cfu	1.30 ± 0.31	71.80 ± 8.51 [a]	51.42 ± 11.58 [d]
	(+ 78.08)	(+ 203.46)	(+ 91.01)
B + T. harzianum @ 8×10^9 cfu	1.55 ± 0.28[c]	74.16 ± 13.29 [a]	52.26 ± 10.78 [d]
	(+ 112.32)	(+ 213.44)	(+ 94.13)
A + Aesofoetida @ 200 ppm/ml	1.03 ± 0.56	20.54 ± 2.35	47.62 ± 5.01
	(+ 41.09)	(- 13.18)	(+ 76.89)
B + Aesofoetida @ 200 ppm/ml	1.28 ± 0.53	51.16 ± 15.56 [d]	42.44 ± 4.43
	(+75.34)	(+ 116.22)	(+ 57.65)

Values in parenthesis are showing the percentage increased over untreated control. Columns bearing superscript are statistically significant ($p<0.05$ LSD) with respective control.
a = $p<0.0001$, b = $p<0.001$ and c= $p<0.01$, d = p<.05.

4. Discussions

Soil fertility is diminishing gradually due to soil erosions, loss of nutrient, accumulation of salts and other toxic elements, water logging and un-balanced nutrient compensation. Organic wastes and bio-fertilizers are the alternate sources to meet the nutrient requirement of crops and to bridge the future gaps. Many efforts are being exercised to combat the adverse consequences of chemical farming [13]. Microbial inoculation by plant growth promoting rhizo-bacteria (PGPR) are naturally occurring by soil bacteria that aggressively colonize plant roots and benefit plants by providing growth promotion. They help in promoting free-living nitrogen-fixing bacteria, increase supply of other nutrients, such as phosphorus, potassium, sulphur, iron and copper, produce plant hormones, enhance other beneficial bacteria or fungi [14, 15]. Using various agricultural by-products as an organic nutrient source for plants due to increasing environment-related issues, as well as the need to dispose of and use rising amounts of waste [16]. Recycling organic waste including dung of dairy cattle, poultry waste and animal litter are used as main source of organic matter for supply of essential minerals needed to plants [17]. A key advantage for using composted material is its potential to resist against root diseases [18].

Suitable growing substrates are essential for quality flower production as these affect development and maintenance of plant rooting system [19]. Now a days, coco coir/ coco peat which is a horticultural by-product obtained after extraction of fiber from coconut husk [20], is in high demand to be used as substrate for production of various floricultural crops. There are also economic benefits, as the use of residues means lower costs than those of conventional materials [20]. The use of compost as organic fertilizer allows improvement in fertility, in addition to being excellent soil conditioner, improving their physical, chemical and biological characteristics, such as retention water, aggregation, porosity, increased the cat ion exchange capacity, increased fertility and increased life soil microbial activities, however the value of compound fertilizer depends on the material used as raw material [21, 22].

Soil is a heterogeneous habitat of beneficial, plant pathogenic and saprophytic microbes, which plays an important role in the spread of soil borne diseases [23]. Several nonchemical methods including addition of organic amendments are an effective method for controlling soil borne pathogens and diseases in various field crops [24, 25]. Similarly, asafoetida or hing, a dry latex or resinous gum from *Ferula asafoetida* has been widely used in various indigenous systems of medicine in India and Pakistan. From old ages, farmers in Malir, Karachi area of Sindh, are applying asafoetida for preventing the plants from root diseases particularly nematode attacks and increasing yield. Soil amendment with asafoetida in general showed suppressive effect on soil borne pathogens and have a positive effect on growth of watermelon both in field plots and at farmer's field [9].

The results showed significant increase in root lengths when treated with coir in combination with Trichoderma and Asafoetida as compared to control. While remaining treatments also promoted the root length non-significantly as compared to control. The possible reason may be the fertilizers added during crop rising. The results are in conformity with those reported by [26] in calendula, who obtained highest plant spread by the application of different growing media. The results are in conformity with those reported by [27] and [28]. It is reported that *Trichoderma* spp. stimulates plant growth in different mechanisms [29].

The most of the treatments significantly promoted the plant shoot length in experimental plants after 90 days in Gladiolus plants. These observations are in line with the findings of Kareem *et al.* (2014) [30]. Where they found that ornamental plants like dahlia showed maximum plant height when grown in compost mixes. The results are also confirmed the findings of Mehmood (2013) [31] who observed increase in number of stems and plant height of *Antirrhinum majus*. This increasing trend in morphological parameters might be due to recommended dose of fertilizer, could be attributed to the quick and readily availability of major nutrients like N, P and K to plants at earlier stages of plant growth. The results obtained in the present investigation are in agreement with earlier findings of Rana *et al*. (2005) [32] and Campbell & Gubbels (1978) [33] in buckwheat, [34].

The coir in both amounts (10 tons/ha and 20 tons/ha) in

combination with both biocontrol agents (Trichoderma and Asafoetida) increased fresh weights of gladiolus plants in present study. All remaining treatments also promote root fresh weights non-significantly. It is reported that *Trichoderma* spp. stimulates plant growth in different mechanisms [35]. Badar & Qureshi in 2014 [36] also reported that composted organic waste increased fresh weights of plants.

Concerning the shoot fresh weights most of the treatments significantly enhanced shoot fresh weights of experimental plants. Maximum promotion seen when experimental plants treated with coir 20 tons/ha in combination with Asafoetida. Only coir in an amount of 10tons /ha alone and Asafoetida @200ppm/ml alone increased fresh weights of Gladiolus plants non-significantly. Badar & Qureshi in 2014[36] also reported that composted organic waste increased fresh weights of plants.

Treatments with Trichoderma alone and in combination with coir 20tons/ha significantly increased root dry weights. The root system is important for plant fitness because it provides anchorage, contributes to water use efficiency and facilitates the acquisition of mineral nutrients from the soil [37]. Coir alone in an amount of 20 tons/ha enhanced maximum shoot dry weights over control after 30 days and in combination with Trichoderma and Asafoetida in both amounts (10 tons/ha and 20 tons/ha) also increased shoot dry weights significantly. The enhanced plant growth by *Trichoderma harzianum* might be due to production of secondary metabolites which may act as an auxin like compound; these materials may lead to the development of the root system and an exploration of a large volume of soil [38 a, b]. Treatment with coir 20tons/ha in combination with Asafoetida significantly promoted number of leaves of Gladiolus plants. Although coir 20 tons/ha alone and in combination with Trichoderma, coir 10 tons/ha in combinations with Trichoderma & Asafoetida non-significantly increased number of leaves of experimental plants. Our findings get support from the previous work done by Wuryaningsih *et al.* (1999) [39] and Waseem *et al*., (2013) [40] who also obtained significant increase in pot anthurium leaf number while using coir dust as growing media. All treatments significantly promoted the leaf area as compared to control after 30 days of experimental plants. Paradiso & de Pascale in 2008 reported maximum leaf area in gerbera when grown in a substrate containing coco fiber along with perlite. Ahmed *et al*., also reported similar results in 2012 [20] on *Gerbera jamesonii*cv. Hybrid Mix.

Coir 20 tons/ha in combination with Trichoderma significantly increased total chl. content of experimental plants. *Trichoderma*-treated plants were able to enhance nutrient uptake, resulting in increasing root and shoot growth, and improving plant vigor to grow more rapidly and to enhance plant greenness, which might result in higher photosynthetic rates [41].

Trichoderma harzianum @ 8×10^9 cfu alone and in combination with coir (both amounts 10 tons/ha and 20 tons/ha) promoted protein content significantly of experimental plants. Asafoetida @200ppm/ml alone and in combination with coir 20tons/ha also increased protein contents of gladiolus plants. The results obtained demonstrated that proteins content of shoots and roots of maize plants treated with *T. harzianum* T22 were increased, these results may attributed to *Trichoderma* spp. increase uptake of nitrates and other ions [42, 43]. Trichoderma and Asafoetida as biocontrol agents alone significantly increased protein content of Gladiolus plants. Improved plant growth might be due to increased solubility of insoluble plant nutrients by *Trichoderma* species [44]. These beneficial effects on plant growth in the existence of *Trichoderma* inoculants are reported due to the enhancement in mineral uptake, decomposing organic matter, production of plant hormones, enzymes and antibiotics. Organic fertilizers and biofertilizers increased protein content of experimental plants [45].

Trichoderma as biofertilizer in combination with coir in both quantities (10 tons/ha and 20 tons/ha) significantly increased carbohydrate content of experimental plants while coir in an amount of 10 tons /ha was also enhanced % carbohydrate of gladiolus plants at pre flowering stage [45]. In 2015, Badar *et al*., reported that composted agro wastes applications as organic fertilizers increased the carbohydrate contents of chickpea plants. Both organic and bio fertilizers increased maximum content of carbohydrate in experimental plants [46].

5. Conclusion

Nutrients availability plays a vital role for healthier growth and production of floriculture crops. In present study addition of organic matter (coconut coir) alone and in combination with microbial inoculants (*Trichoderma harzianum)* as organic amendment caused beneficial effects on physical and biochemical parameters of *Gladiolus grandiflorus.* Application of coconut coir may improve soil quality and availability of nutrients to plants. Improvement of plant growth by *T. harzianum* might be due to increased solubility of insoluble plant nutrients by Trichoderma species.

Soil amendment with asafoetida in general showed suppressive effect on soil borne pathogens and have a positive effect on plant growth. Using coconut coir alone and in combination with microbial inoculants as organic amendment recommended to improving growth of cut flower plants.

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