



## Improvements In Gladiolus Production Technology; Enhanced Gladiolus Production By Treating With Quorum Quenching Bacterial Consortia On Descaled And Cut-Half Gladiolus Corms

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**Abstract:** Here, we compared the impact of quorum quenching based bacterial bio-product in combination with three different sowing methods for gladiolus corms: Scale (S), Descale (DS) and Descale and Cut half (DSC) into portions. Data were recorded at 1<sup>st</sup>, 3<sup>rd</sup>, 6<sup>th</sup> leaf stage for flower emergence. Number of corms harvested showed that the quencher bacterial consortia treated with descaled and cut half type corms, produce more flowers and corms. It also showed more variation in terms of flower emergence. Indeed, the treated plants remained healthier and showed lesser disease index. This study proposes an eco-safe novel bio-product and validates an improved sowing method for sustainable gladiolus flower and corms production while keeping the profitability.

[Sahar JAMEEL, Kashif RIAZ, Tahira Bano, Hassan Zafar Khari, Farhat Naz, Qurat Ul Ain Nazeer, Jahanzeb Ahmad, Hira Aslam. **Improvements In Gladiolus Production Technology; Enhanced Gladiolus Production By Treating With Quorum Quenching Bacterial Consortia On Descaled And Cut-Half Gladiolus Corms.** *Life Sci J* 2021;18(2):51-54]. ISSN: 1097-8135 (Print) / ISSN: 2372-613X (Online). <http://www.lifesciencesite.com>. 8. doi:[10.7537/marslsj180221.08](https://doi.org/10.7537/marslsj180221.08).

**Key words:** Scale (S), Descale (DS), Descale and Cut half (DSC) Introduction

Gladiolus is an important cut flower holding a noticeable position in the international flower market. Its name is derived from a Latin word *gladius* which means sword to it is also known as “sword lily” due to shapes of its leaves. It belongs to family *Iridaceae* and has almost 260 species (Shaukat *et al.*, 2013). It is mostly cultivated in temperate regions of the world and is grown from circular symmetrical corms, it grows in wide range of soils from clay loam to organic rich soil (Misra and Singh, 1989). In the world, United States, Holland, Italy, France, Poland, Bulgaria, Brazil, India, Australia and Israel are among the leading producers of gladiolus. The international flower trade is about US\$11 million per year, for which cut flower contribute about 60%, and this contribution is about to double till 2025 (Arora *et al.*, 2006). In Pakistan, it is the most sought flower after roses. Different ecological areas of Pakistan with temperate climate characteristics such as Pattoki, Chunan, Okara, Kallarkhar, Rawalpindi, Faisalabad, Narowal, Sahiwal, Gurjanwala, Peshawar, Swat, Mirpur and Rawalakot have greater gladiolus production potential (Shaukat *et al.*, 2013). Usually it is grown in three different sowing methods i.e. scale (corms with scales) descale (corms without scales) and

descale and cut half (corms without scale and cut into two halves).

Microbial population present in rhizosphere includes bacteria, fungi, archaea etc, in rhizobacteria important function such as soil and plant health maintenance is controlled through Quorum sensing QS, (Chen *et al.*, 2002). It occurs between same as well as different spp. of bacteria, a cell density dependent signal mediated process that regulate gene expressions in response to fluctuations in cell density population (Riaz, *et al.*, 2008). Signals are called auto inducers or pheromones after establishment of signals recognition of cell density occurs and specific gene expression produces and attack on host plant, in nature several mechanisms do exist that tend to inhibit these sensing process which is called Quorum Quenching, QQ (Dong *et al.*, 2002). It can be done by signal degradation and/or interfering with signal production, perception (Dong *et al.*, 2002). It is therefore important to have a look on the structural and functional properties of these bacterial populations in terms of QS and QQ. Hence this study aims at to improve gladiolus production by using bacterial quorum quenching consortia.

## Material And Methods

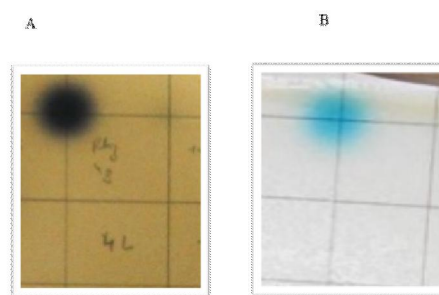
Experimental sites was established on a total area of 1982.88 square feet, ridges were prepared on a total of 25 -15 cm in size, corm to corm distance was 5-10 cm and land was well prepared. Manually sowing of corms was done in 8 cm depth on ridges, 3 treatments was used i.e. scaled, descaled and cut half. Sowing date was 20 November 2014, Irrigation was applied after sowing. 576 corms was sown in this area of 1982.88 square feet. It was decided to use three treatments which was scaled, descaled and descaled and cut half corms.

Scaled corm was the corm with its scale, descaled was the corm without scales, scales was removed manually from corms, in descaled and cut half scales was removed from corms and corm was half cut by a sharp knife. 10 plants were used for the control purpose, these plants were sown in the pots and same soil was used in the pots as used in the field. Pots were placed about 20 feet away from the field. Microbial consortia was treatment. Qq consortia was prepared by the addition of 5 Quorum quenchers bacteria that was isolated from gladiolus field. Mixture was prepared in one day old liquid culture then it was given to the plants by irrigation at 3<sup>rd</sup> and 5<sup>th</sup> leaf stage. Collection of data was done at different stages 1<sup>st</sup>, 3<sup>rd</sup>, 6<sup>th</sup> and 9<sup>th</sup> leaf stage of plant. Length of plant and number of leaves were counted.

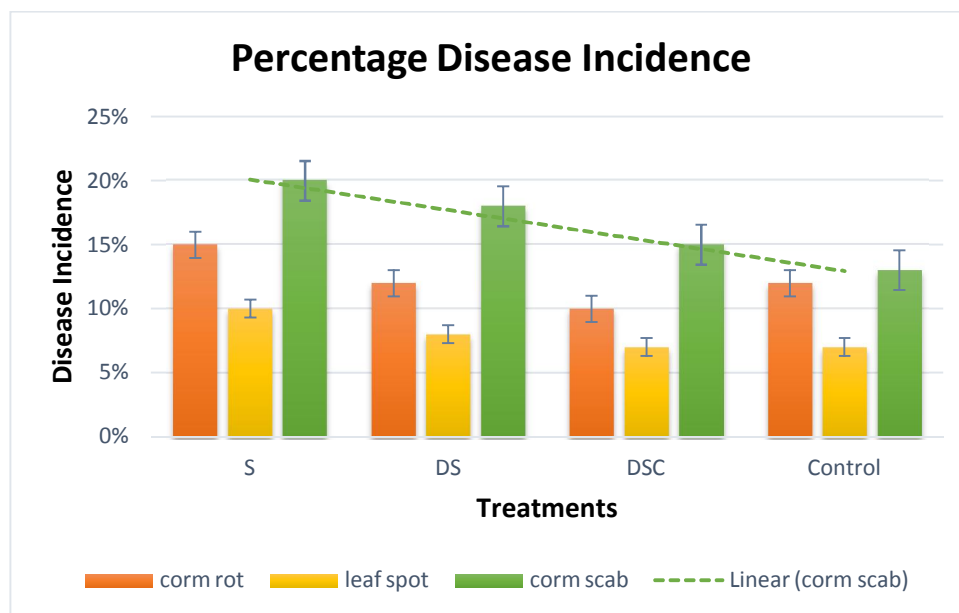
## Data Collection

Quorum quenching bacteria were identified by using biosensors. Different gram negative bacteria synthesises distinct acylated homoserine lactones like opportunistic pathogen *Chromobacterium violaceum* synthesises an N-hexanoyl homoserine lactone (C6-

HSL) (McLean *et al.*, 1997). And plant pathogenic *Agrobacterium tumefaciens* synthesises N-octanoyl homoserine lactone (C8-HSL) (Cha *et al.*, 1998). The *Chromobacterium violaceum* have been used for the detection of QSIs (McLean *et al.*, 2004). This QSIs strain incorporated into a soft agar and streaked in close proximity to the test material. Potential loss of pigmentation of purple violecein production observed which shows the Quorum Sensing inhibition occurs. N-acyl homoserine lactones (NAHLs) were extracted from overnight culture by adding equal amount of ethyl acetate and after shaking the free supernatant was deposited on TLC plates and overlaid with soft agar seeded with biosensors. Quencher strain were added into NAHL having Lauria Beratni medium (LBm) and placed over night then 20 ml solution was taken from tubes and spotted on TLC plates overlaid with soft agar seeded with biosensor. Presence of spot shows strain activity and absence of spot shows inactivity. Production of violet color shows presence of CV026 strain and blue pigmentation shows NTLR4.



QS and QQ activity determination through Biosensor based assay: A: CV026 B: *A. tumefaciens* NTLR4



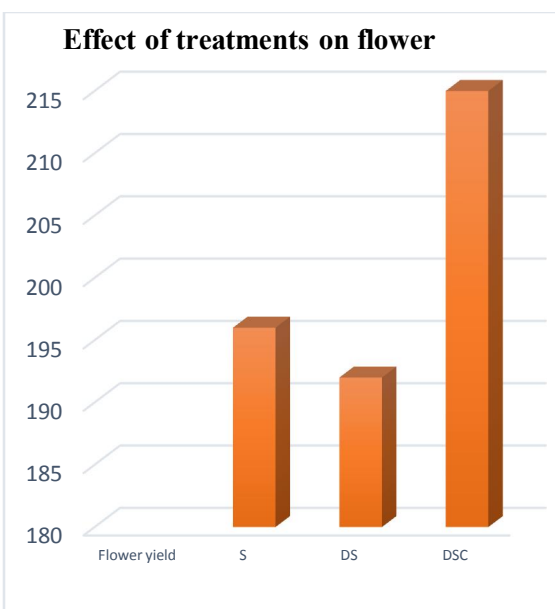
Harvesting were done on the 30<sup>th</sup> of March 2015. Flower harvesting was done when first or second flower is about to open and was done in cool morning and in evening. Sharp knife was used and cutting was done between stem and leaves.

### Results

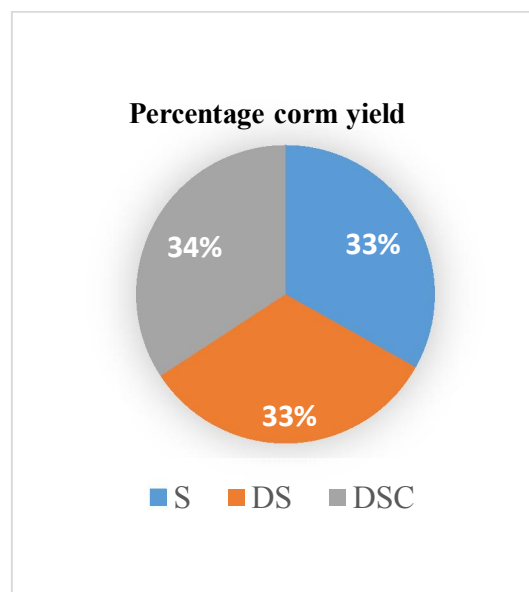
More than 95% of the plants were healthy in the field but all the plants in control got infected with *Burkholderia gladioli*. Application of microbial consortia mixture shows quencher activity and inhibits production of QS bacteria and as a result plants remains healthy. Application of consortia was not applied to the control experiment and it shows 100% disease and yield losses. Quencher consortia not only effectively reduced the disease impact on gladiolus but also enhanced growth parameters.

Three treatments were used i.e. scaled, descaled, descaled and cut half.

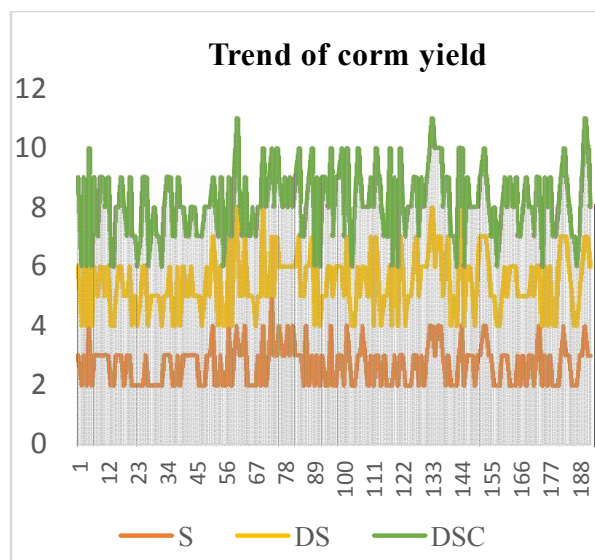
Descale and cut half shows maximum flower yield as compared to the Scale and Descale.



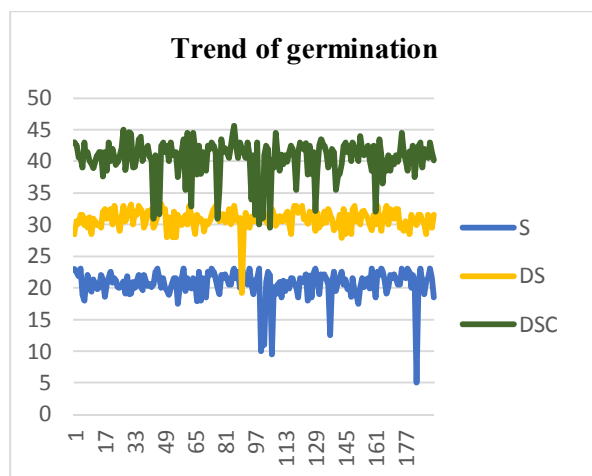
DSC treatment shows more corm yield as compared to the S and DS. With respect of percentage DSC shows 34% of yield whereas both S and DS gives 33% yield.



Scale shows less variation in yield whereas Descale and cut shows more variation.



Trend of germination was also very fascinating as very small difference in trend of germination of S treatment as compared to DS and DSC. DSC shows maximum trend of germination.



### Summary

Gladiolus is an important cut flower after roses, the international flower trade is about 11US\$ million per year on which gladiolus contributes about 60% and this contribution is about to double till 2025. Bacteria have different defence strategies to protect itself as well as to disarm competitors to colonize nutrient and energy rich places by efficiently interfering with the key components of QS mechanism process called Quorum Quenching (QQ)

Three treatments scaled, descaled and descaled and cut half corms were used. QQ consortia was prepared by the addition of 5 Quorum quenchers bacteria that was isolated from gladiolus field. Consortia was prepared from one day old liquid culture then it was given to the plants by irrigation at 3<sup>rd</sup> and 5<sup>th</sup> leaf stage. Foliar spray of microbial consortia mixture shows quencher activity and inhibits production of QS bacteria and as a result plants remains healthy. Quencher consortia not only effectively reduced the disease impact on gladiolus but also enhanced growth parameters. Trend of germination was very fascinating as very small difference in trend of germination of S treatment as compared to DS and DSC. DSC shows maximum trend of germination. More variation in germination were preferred to the farmers because it gives more variation in biological parameters in terms of time while observing the local market trend. Descale and cut half shows maximum flower yield as compared to the Scale and Descale. DSC treatment shows more corm yield as compared to the S and DS. With respect of percentage DSC shows 34% of yield whereas both

S and DS gives 33% yield. Scale shows less variation in yield whereas Descale and cut shows more variation. Descale and cut corm sowing method could be proposed to the farmers because it gives more variation in biological parameters in terms of time while observing the local market trend.

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