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Biofertilizers on Improving Bunch and Berry Attributes in Grapes

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ABSTRACT: An experiment was conducted at Coimbatore to study the effect of biofertilizers on the bunch and berry attributes of grapes. The biofertilizers *viz.*, *Azotobacter*, phosphate solubilizing bacteria (PSB) and potash releasing bacteria (KRB) were applied to the individual vines at three different doses of 100 g, 200 g and 300 g each along with 75% and 100% RDF and control (100% RDF alone). Application of 300 g each of *Azotobacter,* phosphate solubilizing bacteria, potash releasing bacteria with 100% RDF registered positive impact on the bunch and berry attributes of grape *viz.,* maximum number of bunches (32.05), bunch length (20.72 cm), bunch circumference (33.87 cm), bunch weight (275.81 g), berry length (17.49 mm), berry width (16.64 mm) and berry volume (4.12 cm^3). The application of biofertilizers also positively influenced total chlorophyll, total carbohydrate content, nitrate reductase activity, leaf petiole nutrient content, available nutrient and microbial population in the soil.

Keywords: *Azotobacter*, PSB, KRB, Grapes, bunch, berry, attributes.

INTRODUCTION

Grapes (*Vitis vinifera* L.) is one among the few fruit crops which requires special and constant care throughout the cropping period especially from pruning to harvest. The fruits are rich source of sugars, vitamins (C, A, K and B complex), minerals and antioxidants and are continuously in demand for their appeal, flavour, sweetness and nutritional values. Though, grape originated in temperate region near Caspian Sea, in India it is commercially cultivated in tropical and sub-tropical climatic conditions and of which 94% is contributed by tropical regions. Grape is cultivated in an area of 155.3 thousand ha in India with production and productivity of 3,357.68 thousand MT and 21.62 MT/ha respectively. During 2020-21, India earned foreign exchange of Rs. 2,302.16 crores through export of 263,075.67 MT of grapes to the world countries (APEDA, 2022). Production of high-quality grapes is determined by medium sized bunch with good berry size and superior quality, which are in turn influenced by genotype, climate and horticultural practices *viz.*, training, pruning, nutrition management and growth regulator application. Grapes is highly responsive to nutrient application and vine growth, productivity and quality are highly influenced by the quantity of nutrients made available to the vine. The excessive usage of inorganic fertilizers in grapes for maximizing yield has hassled soil and environment (Wu *et al.,* 2020). Application of organic manures and biofertilizers will aid in improving the soil health by tremendous increase in microbial population and in turn enhancing the availability of nutrients to the plants by improved microbial activity. In addition, incorporation of biofertilizers alter soil texture, structure and aeration and thereby influence the growth and yield of plants (Sharma *et al.*, 2012). Soil application of biofertilizers along with recommended organic manures is one of the natural ways of improving productivity that lead to sustainable and ecofriendly cultivation of horticultural crops. Hence, this study was carried out to evaluate the influence of biofertilizers *viz.*, *Azotobacter*, phosphate solubilizing bacteria (PSB), potash releasing bacteria (KRB) on the bunch and berry attributes of grapes.

MATERIALS AND METHODS

The experiment was conducted in the farmer's field at Coimbatore, on eight years old grapevine cv. Muscat Hamburg, grafted on Dog Ridge rootstock with a spacing of 4 m × 2 m. The vines were trained on bower system. Soil type of the experimental vine yard was sandy loam with pH, EC, available N, P and K of 7.67, 0.08 dS/m, 224 kg/ha, 38 kg/ha and 199.5 kg/ha respectively. The grapevines of mean girth 12±0.9 cm was selected for the study and the experiment was laid out in Randomized Block Design, consisting of seven treatments with three replications *viz.*, T_1: 100% RDF (200:160:600 g NPK/vine) (control), T_2: 100% RDF + 100 g *Azotobacter* + 100 g PSB + 100 g KRB/ vine, T_3: 100% RDF + 200 g *Azotobacter* + 200 g PSB + 200 g KRB/vine, T_4: 100% RDF + 300 g *Azotobacter* + 300 g PSB +300 g KRB/vine, T_5: 75% RDF + 100 g *Azotobacter* + 100 g PSB +100 g KRB/ vine, T_6: 75%

RDF + 200 g *Azotobacter* + 200 g PSB + 200 g KRB/vine and T_7: 75% RDF + 300 g *Azotobacter* + 300 g PSB + 300 g KRB/vine. The recommended dose of fertilizers for the (delete space) cv. Muscat Hamburg was 30 kg FYM and 200:160:200 g NPK/vine. The biofertilizers *viz*., *Azotobacter*, PSB and KRB procured from the Department of Agricultural Microbiology, Tamil Nadu Agricultural University, Coimbatore were used for the study. Immediately after pruning of the grapevines, the biofertilizers were applied as per the treatments along with well decomposed farmyard manure @ 30kg/vine. The inorganic fertilizers *i.e.,* full dose of recommended nitrogen and phosphorus and half the dose of recommended potassium were applied two weeks after the application of biofertilizers. The remaining half of the recommended quantity of potassium was applied 60 days after pruning as top dressing.

Observations were recorded on bunch and berry attributes *viz*., bunch length (cm), bunch circumference (cm), bunch weight (g), berry weight (g), berry length (mm), berry width (mm), berry volume (cm^3) and number of bunches per vine. The length of each bunch was measured from proximal end to distal end of bunch stalk with the flexible measuring tape and the mean was worked out and expressed in centimeters. The circumference of the bunch was recorded at the middle portion of the bunch to arrive at the mean and expressed in centimeters. Bunches collected from the randomly tagged vines were weighed and computed for mean bunch weight and expressed in grams. The weight of hundred randomly selected berries were recorded and divided by hundred to arrive at the mean and expressed in grams. The length of berry was measured from the bottom to the tip of the berry using digital vernier caliper and expressed in millimeter and the width was measured at the center point of maximum width and expressed in millimeter. The volume of the sampled twenty berries per replication per treatment was determined by water displacement by the berries and expressed in cubic centimeter and then the average volume of the berry was worked out. The number of bunches per vine was counted on each vine and the mean value was worked out.

Observations were recorded on physiological parameters such as the chlorophyll content, total carbohydrate content, nitrate reductase activity, N, P and K content in the leaf petiole. The chlorophyll a, chlorophyll b and total chlorophyll was determined in the leaf present opposite to the inflorescence at flowering stage by acetone method (Yoshida *et al*., 1971). The total carbohydrate content of the canes (collected after harvest) was estimated as given by Somogyi (1952). Nitrate reductase activity was calculated from the leaf borne opposite to the inflorescence collected at bud initiation stage as described by Nicholas *et al.* (1976).The nutrient content N, P and K in the petioles of the leaves present on opposite side to the inflorescence were analysed. The total nitrogen content was analyzed by using diacid extract and Micro Kjeldahl method (Humphries, 1956), the total phosphorus was analyzed after extraction triple acid adopting Vanadomolybdate phosphoric yellow colour method and the triple acid extract also used to estimate potassium by using flame photometer (Jackson, 1973).

Soil samples were taken from the vine yard before and after imposing the treatments. The available nitrogen in the soil was analyzed by alkaline permanganate method (Subbiah, 1956), the available phosphorus by using the principle of Olsen *et al.* (1954) and the available potassium after extraction with neutral normal ammonium acetate, using flame photometer (Stanford and English 1949). The total bacterial, fungal and action bacteria population in the rhizosphere soil were estimated by pour plate method (Allen, 1957). Data were analyzed using one-way ANOVA and Critical Difference (CD) was used to separate significant differences between means with the help of a statistical software package, SPSS v.20.00.

RESULTS AND DISCUSSION

The continuous and indiscriminate application of inorganic fertilizers for enhancing productivity in crop plants resulted in deterioration of soil health, reduced soil fertility and caused environmental degradation. Application of biofertilizers is an ecofriendly approach that is used extensively in crop production for improving soil health, enhancing nutrient availability and regulating contamination in the environment caused by excessive application of inorganic fertilizers to a certain extent (Sharma *et al*., 2012). An integrated approach of combining organic inputs and biofertilizers along with judicious application of inorganic fertilizers is gradually employed in sustainable crop production.

Application of biofertilizers along with organic manures enrich the availability of nutrients in the soil which in turn increases the ability of the plants to uptake the solutes from the rhizosphere. In the present study, with respect to bunch attributes, the number of bunches, bunch length, bunch circumference and bunch weight were found to be superior in the treatment receiving 100% RDF along with 300g each of *Azotobacter*, PSB and KRB (Table 1). The increased bunch weight might be due to improved internal nutrient status of the vine leading to increased growth and vigour and further have contributed for the increased yield per vine. Better performance for berry attributes in the same treatment might be due to the supply of optimum nutrients during the entire crop growth, which led to sufficient vegetative growth resulting in more photosynthesis (Singh and Varu 2013; El-Mahdy *et al*., 2017). *Azotobacter* is responsible for the dry matter accumulation and translocation apart from fixing nitrogen in the soil and also produce growth regulators like IAA, GA_3 and cytokinins. The biofertilizer application in addition to improving the nutrient availability to the plant root system also aids in translocation of assimilates from source to sink (Sharma and Sharma 2006). The optimum supply of macro and micronutrients and growth regulating substances to the grapevines receiving 100% RDF along with 300g each of *Azotobacter*, PSB and KRB resulted in better filling of berries; this might be due to uptake of nutrients in a well-balanced manner which paved way for better metabolic activities and thereby led to increased protein and carbohydrate synthesis. In addition, *Azotobacter* with the ability to release phytohormones and partitioning of photosynthates towards the sink might have led to increased berry size and weight. These results are in line with the findings of El-Mahdy *et al.* (2017) in grapes cv. Thompson Seedless.

In the present study, significant differences were observed among the treatments for physiological parameters *viz*., total chlorophyll content, nitrate reductase activity and total carbohydrate content ant the treatment receiving 300 g each of *Azotobacter*, PSB and KRB along with 100% RDF was found significantly superior for all the physiological parameters observed (Table 2). Chlorophyll content in the physiologically active leaf is a more correlated measure for the estimation of nitrogen uptake from the soil under different growth conditions. The chief constituent of chlorophyll is nitrogen and its uptake is enhanced through increased availability of nitrogen to the plants. In this study, total chlorophyll content in the leaves increased due to combined application of 100% RDF along with 300g each of biofertilizers, which enhanced the photosynthetic efficiency and ultimately improved the fruit yield (Kundu *et al.*, 2011). Nitrate reductase, an important plant enzyme, involved in the assimilation of exogenous nitrate and its activity gives a good measure of the nitrogen level in the plant and is often correlated with plant growth and yield. A notable increase in NRase at higher dose of biofertilizers was also observed in earlier studies (Tobar *et al.*, 1994) and the result for nitrate reductase activity in the present study was found to be in line with earlier work. Carbohydrates which get assimilated are translocated efficiently towards the developing bunches (sink) and tend to increase the yield. The increased leaf nitrogen content resulting in enhanced photosynthesis might have increased the proportion of carbohydrates and the results obtained is in line with Stino *et al.* (2009).

The optimum amount of nutrients is essential to ensure proper flowering, fruit set and yield in grapes. Petiole analysis during flowering stage is the reliable method to ascertain the grapevine nutritional status. The excess or deficit level of a particular nutrient in the petiole is a reflection of its real status in the soil. Singh and Sharma (1993) reported that application of biofertilizers along with chemical fertilizers enhanced the concentration of leaf nutrients particularly nitrogen and phosphorus in sweet orange. In the present study, with respect to leaf petiole nutrient status, the maximum nitrogen, phosphorus and potassium contents were registered by vines receiving 100% RDF and 300g each of biofertilizers (Fig.1a). Moreover, by increasing the dosage of biofertilizers, the solubility and availability of the required plant nutrients improved which in turn enhanced the levels of leaf NPK content in grapes. Similar results were reported in grapes and mango by El-Sabagh *et al.* (2011); Sau *et al.* (2017) respectively. Addition of organic manures might have reduced the loss of nutrients through leaching from the soil and enhanced soil microbial population.

Table 1: Effect of biofertilizers on bunch and berry attributes in grapes.

Treatments	Bunch length (cm)	Bunch circumference(cm)	Bunch weight(g)	Bunches per vine	Berry length (mm)	Berry width (mm)	Berry weight(g)	Berry volume (cm^3)
T_1	18.2	29.32	259.40	22.90	16.81	15.85	3.17	3.92
T_2	17.12	28.45	250.54	25.50	16.52	15.79	3.13	3.76
T_3	19.03	31.92	264.64	29.46	16.96	16.09	3.38	4.02
T_4	20.72	33.87	275.81	32.05	17.49	16.64	3.87	4.12
T_5	15.94	27.11	240.11	23.76	15.71	15.26	2.69	3.25
T_6	17.04	28.05	245.17	24.68	16.70	15.83	3.14	3.84
T_7	19.01	31.45	260.96	26.92	16.89	15.97	3.30	4.00
SEm±	0.14	0.23	1.94	0.48	0.13	0.08	0.08	**0.10**
CD (0.05)	**0.78**	**1.36***	**11.16***	**2.76***	**0.76**	**0.47***	**0.43***	**0.23***

*Significance at 5 percent level

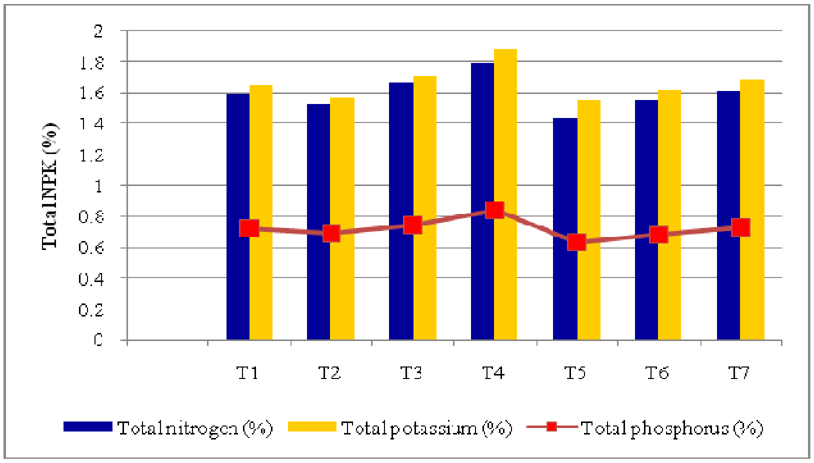
Table 2: Effect of biofertilizers on physiological parameters in grapes.

Treatments	Chlorophyll a (mg/g)	Chlorophyll b (mg/g)	Total chlorophyll (mg/g)	Total carbohydrate content (%)	Nitrate reductase Activity (µg NO_2/g/h)
T_1	1.73	1.04	3.85	30.04	32.26
T_2	1.67	0.99	3.12	29.45	31.82
T_3	2.05	1.11	4.15	30.83	45.01
T_4	2.23	1.14	4.65	32.46	50.14
T_5	1.27	0.79	2.02	23.77	27.60
T_6	1.68	0.98	3.79	30.00	31.93
T_7	1.98	1.05	3.98	30.04	33.13
SEm±	0.02	0.01	0.03	0.21	0.70
CD (0.05)	**0.08***	**0.04***	**0.21***	**1.23***	**4.01***

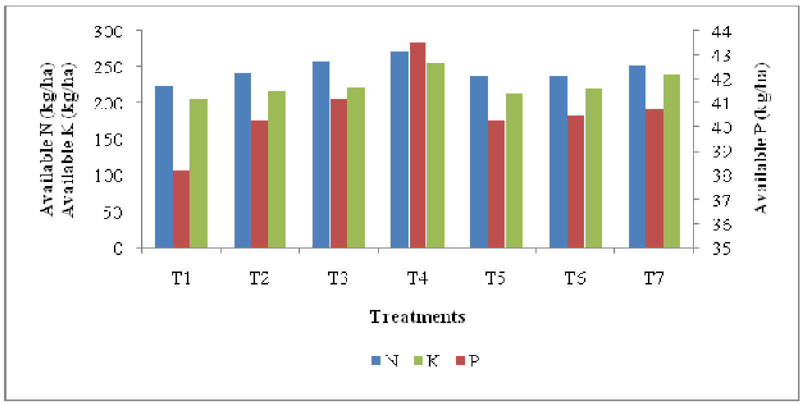
*Significance at 5 percent level

Treatment details

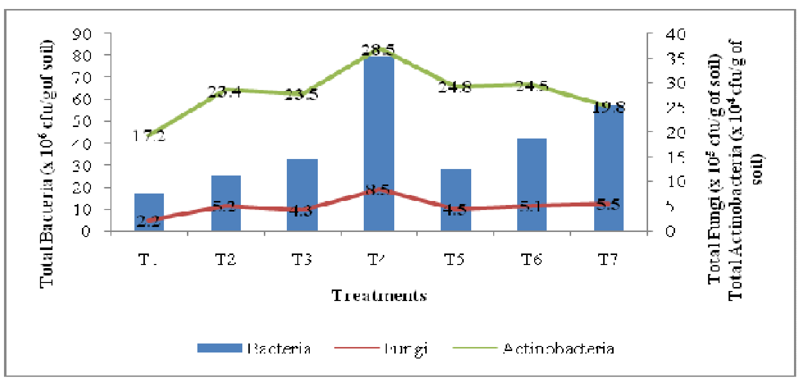
T_1- 100% RDF (200: 160:600 g NPK/vine) (control); T_2 -100% RDF + 100 g *Azotobacter* + 100 g PSB + 100 g KRB/ vine; T_3-100% RDF+ 200 g *Azotobacter*+ 200 g PSB + 200 g KRB/vine; T_4-100% RDF+ 300 g *Azotobacter*+ 300 g PSB + 300 g KRB/vine; T_5-75% RDF + 100 g *Azotobacter* + 100 g PSB + 100 g KRB/ vine; T_6-75% RDF + 200 g *Azotobacter*+ 200 g PSB + 200 g KRB/vine; T_7-75% RDF + 300 g *Azotobacter*+ 300 g PSB + 300 g KRB/vine



(a) N, P and K content in leaf petiole.



(b) Available N, P and K content in the soil.



(c) Total microbial count in the soil.

Treatment details

T_1- 100% RDF (200: 160:600 g NPK/vine) (control); T_2 -100% RDF + 100 g *Azotobacter* + 100 g PSB + 100 g KRB/ vine; T_3-100% RDF+ 200 g *Azotobacter*+ 200 g PSB + 200 g KRB/vine; T_4-100% RDF+ 300 g *Azotobacter*+ 300 g PSB + 300 g KRB/vine; T_5-75% RDF + 100 g *Azotobacter* + 100 g PSB + 100 g KRB/ vine; T_6-75% RDF + 200 g *Azotobacter*+ 200 g PSB + 200 g KRB/vine; T_7-75% RDF + 300 g *Azotobacter*+ 300 g PSB + 300 g KRB/vine

Fig. 1. Effect of biofertilizers on leaf nutrient content, soil nutrient content and total microbial population in grapes.

Application of biofertilizers may result in increased availability of essential nutrients by converting insoluble forms to soluble and available forms to the plants (Alexander, 1977). During mineralization, microbes convert organically bound nitrogen to inorganic form resulting in higher available nitrogen (Tolanur and Badanur 2003; Sebastiao *et al.*, 2018).

The potash solubilizers speed up the process of mineralization of insoluble K and structurally unavailable forms of K compounds by mobilizing and solubilizing through the secretion of various types of organic acids. Huadan *et al.* (2020) reported that in grapes, application of biofertilizers increased the enzyme activity in the soil and thereby increased the availability of phosphorus which in turn promoted growth and quality of grapes. The improvement in bunch weight and yield might be due to the better nutritional status in the leaf petiole and availability of nutrients in the soil.

Organic residues in the soil will undergo microbial decomposition and release organic acids and other products of decay and will also act as strong binding agents in the formation of large stable aggregates. Addition of organic manures would have resulted in increased secondary and micronutrients availability in the soil which might have aided in increase of microbial load (Rajalingam, 2000). Besides, nitrogen fixation, *Azotobacter* is reported to produce thiamine, riboflavin, nicotine, IAA and gibberellin which regulate the plant growth. Significant differences were observed in soil microbial population among the treatments. Maximum bacterial population (79.64 × 10^6cfu/g of soil), fungal population (8.5 × 10^5cfu/g of soil) and actinobacteria population (28.5 × 10^4 cfu/g of soil) were recorded in the treatment receiving 100% RDF and 300g each of *Azotobacter*, PSB and KRB/vine (Fig. 1c). Microorganisms present in the soil play vital roles in plant growth and development by improving nutrient availability in the rhizosphere. The soil microorganism's exudation modifies the rhizosphere and is an important phenomenon that regulates not only the availability of nutrients in the soil but also their absorption by plants. The microbial population of the soil determines soil health and soil nutrient status. The presence of larger microbial population is the indication for the good soil health which in turn enhances the required nutrient availability to the plant and improve the plant nutrient uptake leading to increased crop productivity. This result is in corroboration with Huadan *et al.* (2020) in grapes.

CONCLUSIONS

The results from the present investigation indicated that application of 300 g each of *Azotobacter,* phosphate solubilising bacteria and potassium releasing bacteria along with 100% RDF /vine registered better performance for bunch and berry attributes in grapes cv. Muscat Hamburg.

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Conflict of interest. None.

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