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Response of Squash Plants (*Cucurbita pepo* L.) to Foliar Spray with Methylo-trophic Bacteria, Methanol and their Combination under Protected Cultivation

Heba H. Mohamed* and Reda E. Ahmed



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Protected Cultivation Dept., Horticulture Research Institute, Agriculture Research Center, Giza, Egypt

ABSTRACT

Two field experiments were carried out during the two successive seasons of 2017/2018 and 2018/2019 at the experimental Farm at Kaha Vegetable Research Farm, El-Kaluobia Governorate Horticulture Research Institute, Agriculture Research Center (ARC), Egypt, under greenhouse. The study aimed to investigate the response of squash to foliar spray with methylo-trophic bacteria ($10 \text{ cm}^3/\text{l}$), methanol (20%) and the combination between them in addition to the control treatment on vegetative growth, productivity and chemical composition of Pera squash cultivar. A complete randomized block design with three replicates was used. The foliar spray treatments were carried out six times during the growing seasons. The first spray was followed after 20 days from transplanting, then others were applied each two weeks. The obtained results indicated that, foliar spraying of the plants with methylo-trophic bacteria plus methanol reflected the greatest values of vegetative growth, fruit yield and its components as well as the physical fruit quality.

Keywords: Squash, Methanol, Methylo-trophic bacteria, Fruit yield..



INTRODUCTION

Squash (*Cucurbita pepo* L.) is a member of the family Cucurbitaceae and it is one of the most popular vegetable crops for human nutrition, not only in Egypt, but also all over the world. Squash is cultivated all over the year in Egypt, in open field during spring and summer, while under in tunnels or greenhouse in fall and winter. According to the statistics of Ministry of Agriculture in Egypt (2019), the total cultivated area was 18670 fed., by an average of 13 tons/fed. The squash fruits are rich of niacin and it has medium values in both riboflavin and ascorbic acid content, it has also low calories. The squash fruit contains 95 % water, 0.9 % protein, 1 % fat, 1 % sugars, 2 % starch, 0.5 % fibers, 0.54 % ash, 18 mg Ca, 0.35 mg Fe and moderate amount of vitamin A. Although the plants are grown under the greenhouse, they are exposed to cold, which affects the yield and the quality of the fruits, the intensity of extreme weather have a different effect of plants at molecular function, morphological characteristics, physiology and developmental processes. In this concern, there is an urgent need to improve the agricultural practices to ensure that crop production is balanced with environmental sustainability (Gray and Brady (2016)). Foliar spray of some growth stimulants considered as one of the most important points among management strategy to improve growth, flowering, yield and quality of the horticultural crops particularly under stress conditions. Growth stimulants are micro-organisms or substances supplemented for plants to enhance the tolerance of the abiotic stress, nutrient uptake and efficiency, as well as crop growth, quality and productivity. Spraying growth stimulants such as, methylo-trophic bacteria to overcome the effects of undesirable conditions are needed.

Methylobacterium spp. are a group of bacteria known as pink-pigmented facultative methylo-trophs (PPFMs) connected with more than 70 plant species that actively colonize in the different parts of the plants like branches, roots and leaves. Some studies illustrated that *Methylobacterium* spp. are identified as endophytes of different plants, such as citrus fruits, pine, cotton, eucalyptus, strawberries, peanuts, hemp, *Catharanthus roseus*, mangroves and tobacco. Methylo-trophs promote plant growth through beneficial interactions with plants by producing phytohormones and indirectly by enhancing the availability of nutrients. Methylo-trophs colonize in different parts of the plant and produce phytohormones like auxins, cytokinin and zeatin. Plant growth substances promote growth of both shoot and root system. Methylo-trophs are widely used as bio-inoculants as a foliar application on plants and serve as an alternative to chemical fertilizers to increase crop yield (Abd El-Gawad *et al.* (2015) and Ashok *et al.* (2020)). Krishnamoorthy (2020) indicated that Pigmented facultative methylo-trophic bacteria are one of the promising candidates for improving plant growth under stress condition. Generally, all the plant species has PPFM as their microbial partner in the phyllosphere region. Hence isolating the habitat adopted efficient PPFM strains and using them as a bio-inoculant for crop plants may enhance the plant growth and yield. Methylo-trophs application as bio-inoculants and as foliar application to crops is common, and their use as alternatives to chemical fertilizers is also increasing. Methylo-trophs use multiple mechanisms to stimulate plant growth, which makes them a suitable and promising candidate for use in sustainable agriculture Kumar *et al.* (2016). Abo-Sedera *et al.* (2018) found that

* Corresponding author.

E-mail address: heba.hanafy.mk@gmail.com

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using foliar spraying methylotrophic bacteria and methanol at 20% reflected the highest values in growth and yield traits strawberry.

Methanol is one of the substances that increased CO₂ fixation in C3 plants, it can compensate a part of the carbon lost by photosynthesis (Nonmura and Benson, (1992) and Fall and Benson (1996)). Methanol can reduce the production of ethylene in plants and delay leaf senescence. Cellular pectin demethylation is the essential source of methanol production in plants. One of the main pillars of sustainable agriculture is using bio - fertilizers with a goal to substantially minimize the use of chemical inputs Sharma (2002) and enhance sustainability. (Maziar *et al.* (2011), Salehi (2013), Soghani *et al.* (2014) and Moemenpour and Karami (2005)) they found that methanol enhanced growth, productivity and quality of produced fruits. Methanol as a carbon source can be very useful in improving the synthesis in the greenhouses. The foliar spray with Methanol decrease uses of fungicides to control windburn and rust (Heins, (1980) and Zbiec *et al.* (2003)) indicated that methanol foliar spray increased the concentration of carbon in the plant by increases plant growth. Concentration of 20 to 30% methanol gave significant increase in grain yield (Rajala *et al.* (1998);Rowe *et al.* (1994)). Foliar spray of methanol can prevent the reduction in biomass, and increase protein

content of peanuts Wishgahi *et al.* (2007). Moshabaki-Isfahani (2011) illustrated that the use of bio-fertilizers with chemical fertilizers enhanced the fresh and dry weight, plant height, leaf chlorophyll content and the amount of macro and micro nutrients absorbed by plant roots. Therefore, the aim of this investigation was to study the effect of foliar spray of methanol and methylotrophic bacteria on squash growth and fruit yield under greenhouse condition.

MATERIALS AND METHODS

Two field experiments were carried out under a polyethylene 540 m² greenhouse during the two successive seasons of 2017/2018 and 2018/2019 at the farm of Agriculture Research in Kaha, Kaliobia Governorate, Horticultural Research Institute, Agricultural Research Center under greenhouse to study the response of squash to foliar spray with methylotrophic bacteria (10 cm³/l) , methanol (20%) and the combination between them in addition to the control treatment on vegetative growth, chemical composition and productivity of Pera squash cultivar. A complete randomized block design with three replicates was adopted. The soil of the experimental farm was clay loam in texture with pH 7.98 .Soil mechanical and chemical analyses are shown in Table (A).

Table A. Soil mechanical and chemical analyses of the soil

Physical analysis	Chemical analysis				
	Cations meq/l		Anions meq/l		
Coarse sand	8.25%	Ca ⁺⁺	9.65	CO ₃ ⁻	Zero
Fine sand	16.15%	Mg ⁺⁺	3.16	HCO ₃ ⁻	5.38
Silt	24.60%	Na ⁺	6.53	Cl ⁻	5.93
Clay	51%	K ⁺	1.16	SO ₄ ⁻	9.19
Texture class:	clay loam				
Soil pH	7.9	Available N		22.5 mg/kg	
E.C dS/m	2.16	Available P		9.1 mg/kg	
Organic matter	3.1%	Available K		120 mg/kg	

Seeds of Pera cultivar were sown in 84 cell trays (cell diameter 9 mm) containing 26.5 cm³ of peat-based commercial substrate. at October 12th and 14th during 2017/2018 and 2018/2019 seasons, respectively. After 15 days, the seedlings, which were at the two true-leaf stage, were transplanted in greenhouse in October 26 and 28 in the first and second seasons, respectively. Each experimental plot was 18 m length and 1.20 m width. Transplants were spaced 0.5 m apart, on the two sides of the ridge. All common agriculture practices were done according to the recommendation by the Ministry of Agriculture for squash crop.

Methylotrophic bacteria: -Isolates of PPFM (pink-pigmented facultative methylotrophs) were prepared as the technique described by Glickmann and Dessaux(1995), Doronina and Trotsenko(1994) and Fletcher and McCullagh (1971).

Methanol: -Methanol is a commercial product from biochemist for laboratory chemicals. Egypt contains; Assay 99.5%.

The experiment included 4 treatments with methylotrophic bacteria and methanol and the combination as follows: -

- Foliar spray with methylotrophic bacteria at 10cm³/l.
- methanol (CH₃ OH) at 20%.

- the combination between them with the same concentrations.

- control treatment (spray with distilled water).

The plants were sprayed six times during the growing season starting 20 days after transplanting and every two weeks intervals.

Data recorded: -

1. Vegetative growth characteristics.

At the middle of fruit picking stage (50 days from sowing) eight plants were chosen at random from each plot and plant height, number of leaves per plants and leaf area were recorded.

2. Yield and its components:

a. Early fruit yield /plant: It was determined as weight of first four harvested fruits

b. Total fruit yield /plant: It was calculated using plot yield and plot area all over the season then fruit yield per plant was calculated.

3- Fruits quality:

a. Physical quality: A random sample of 10 fruits from each experimental plot was taken to determine fruit weight, length and diameter.

b. Chemical quality:

1. Total soluble solids (T.S.S.):A random sample of 10 fruits from each experimental plot was taken to determine

the percentage of soluble solid content by using the hand refractometer.

2. Ascorbic acid (Vitamin C): It was determined using the indicator of 2,6 dichlorophenol indophenol for titration as the method mentioned in A. O. A. C. (1990).

3. Total sugars: Total sugars were determined by the method described by Nelson (1974).

Statistical analysis:

The obtained data were subjected to statistical analysis by the method of Duncan's multiple range test as reported by Gomez and Gomez (1984). All statistical analysis was performed with SAS computer software.

RESULTS AND DISCUSSION

1. Vegetative growth characteristics: -

Data in Table(1) indicate that all the studied growth parameters i.e. plant height, number of leaves per plant, leaf area and chlorophyll reading of squash plants were significantly increased during the two seasons of growth as a result of foliar spraying with methanol compared with the control treatment. In this connection, using foliar spraying of methanol plus bacteria gave increased plant height, number of leaves per plant and chlorophyll reading without significant differences with spraying methanol only during the two seasons, in addition, using foliar spraying of methylotrophic bacteria gave significantly an increase in chlorophyll reading during the first season.

Table 1. Effect of spray with methylotrophic bacteria, methanol and their combination on vegetative growth and chlorophyll reading of squash plants in 2017/2018 and 2018/2019 seasons.

Treatments	2017/2018				2018/2019			
	Plant Height (cm)	Leaves number /plant	Leaf area (cm ²)	Chloro-phyll reading (SPAD)	Plant height (cm)	Leaves number /plant	Leaf area (cm ²)	Chloro-phyll reading (SPAD)
Methanol	68.76 a	36.19 a	2741.8 b	51.93 a	73.38 a	39.14 a	3131.0 b	54.33 a
Bacteria	57.86 b	30.31 b	1671.1 c	49.63 a	60.50 b	33.86 b	2268.9 c	47.24 b
Methanol + bacteria	68.62 a	38.21 a	3368.3 a	54.06 a	74.39 a	41.33 a	3865.0 a	54.23 a
Control	54.47 b	31.67 b	1673.2 c	43.43 b	55.33 c	32.64 b	1834.2 d	41.13 c

Values in the same column followed by the same letter do not significantly differ from each other according to Duncan's multiple range tests at 5% level.

The lowest values of all vegetative growth parameters was found at the control treatment during the two studied seasons. Such increments in growth parameters may be due to that such micro-organisms increased cytokinin and hormones concentrations in plant leaves which act as plant growth promoters and consequently positively affect physiological processes such as cell division and elongation which in turn affect tissues formation and consequently increased vegetative growth of plant.

2. Yield and its components:

The effects of foliar spray with methanol 20%, methylotrophic bacteria and methanol on fruit weight, early and total yield per plant of squash cv. "Pera" are presented in Table (2). The combined treatment of methanol foliar spray with methylotrophic bacteria plus methanol or methanol only gave significant effects on yield and its components. Plants sprayed with the combined treatment of

methanol plus methylotrophic bacteria increased fruits number compared with untreated plants as control. The highest early and total yield of squash plants were found by spraying with methanol plus methylotrophic bacteria in the two seasons compared to the other studied treatments. In addition, using foliar spray of methanol only significant increment in total yield in the second tested seasons. These results could be attributed to the significant increases in all studied vegetative growth parameters which may be caused some important and multiple physiological function in plant cells and buds during flowering and affect subsequent yield by the stimulating effect of PPFM. The ability of PPFM for producing some growth regulators was confirmed previously by Dhale *et al.* (2010), Ahmed (2011) and Abd El-Gawad *et al.* (2015) they found that methylotrophic bacteria enhanced growth, productivity and yield quality of produced fruits.

Table 2. Effect of spray with methylotrophic bacteria, methanol and their combination on fruit yield of squash plants during 2017/2018 and 2018/2019 seasons.

Treatments	2017/2018			2018/2019		
	Fruit weight (g)	Early yield plant (g)	Total yield plant (Kg)	Fruit weight (g)	Early yield plant (g)	Total yield plant (Kg)
Methanol 20%	82.82 a	738.22 b	2.21 a	90.43 b	892.67 b	2.68 a
Bacteria 10 cm ³ /l	61.88 c	368.11 b	1.10 c	65.40 d	395.77 d	1.31 c
Methanol+ bacteria	87.51 a	825.77 a	2.35 a	92.12 a	947.66 a	2.84 a
Control	74.50 b	465.11 b	1.40 b	76.38 c	511.89 c	1.53 b

Values in the same column followed by the same letter do not significantly differ from each other according to Duncan's multiple range tests at 5% level.

3. Fruit physical characteristics: -

Data in Table (3) show the effect of methanol 20%, bacteria methylotrophic and the combination with them on fruit physical characteristics i.e. fruit weigh, length and diameter of squash plants. Data indicated that the foliar application of methylotrophic bacteria plus methanol

increased significantly fruit weigh, fruit length and fruit diameter of squash in the two seasons compared to the other studied treatments. Also, foliar spraying with methanol only enhanced fruit weigh in the first season. Such increments in fruit length, diameter and weight in case of methylotrophic bacteria and methanol may be due to the effect of such foliar

spray with methylotrophic bacteria plus methanol on water content of fruits which affect cell formation and cell size in fruit receptacle and in turn on fruit parameters. Such results show that these bacteria may be act well by the addition of methanol. These effects might be mediated by the production of synthesis of plant hormones Abo-Sedera *et al.* (2018). Obtained results are in harmony with those reported

by Abd El-Gawad *et al.* (2015) in case of using yeast extract, methylotrophic bacteria and Abo-Sedera *et al.* (2018) who found that spraying strawberry plants with methylotrophic bacteria at 10 cm³/l and methanol at different tested concentrations (5,10,15 and 20%) significantly increased all measured fruit physical parameters compared with the control.

Table 3. Effect of spray with methylotrophic bacteria, methanol and their combination on physical fruit characters of squash plants during 2017/2018 and 2018/2019 seasons.

Treatments	2017/2018			2018/2019		
	Fruit weight (g)	Fruit length (cm)	Fruit diameter (cm)	Fruit weight (g)	Fruit length (cm)	Fruit diameter (cm)
Methanol	82.82 a	10.99 b	3.10 b	90.43 b	10.65 b	3.14 b
Bacteria	61.88 c	10.70 b	2.83 c	65.40 d	10.56 b	3.06 b
Methanol+bacteria	87.51 a	11.88 a	3.34 a	92.12 a	11.60 a	3.40 a
Control	74.50 b	10.50 b	2.83 c	76.38 c	10.60 b	3.06 b

Values in the same column followed by the same letter do not significantly differ from each other according to Duncan's multiple range tests at 5% level.

4. Fruit chemical composition: -

Data in Table (4) cleared that the combined treatment of methanol foliar spray with methylotrophic bacteria plus methanol or methanol only gave the highest total soluble solids, total sugars and vitamin C of squash without significant differences between them in the two tested seasons compared to the other studied treatments. In this respect, Maziaret *al.*(2011), Salehi (2013), Soghani *et al.* (2014) and Moemenpour and Karami (2015) found that methanol enhanced growth, productivity and quality of

produced fruits. Spraying the plants with methylotrophic bacteria and methanol at the different tested concentrations statistically affected fruit contents of total soluble solids and vitamin-C as well as, total sugars compared to the control treatment. In addition, foliar spray of strawberry plants with methylotrophic bacteria at 10 cm³/l followed by methanol at the highest used concentration (20%) gave the highest values for all aforementioned chemical constituents without significant differences among them Abo-Sedera *et al.*(2018).

Table 4. Effect of spray with methylotrophic bacteria, methanol and their combination on chemical fruit quality of squash plants during 2017/2018 and 2018/2019

Treatments	2017/2018			2018/2019		
	T. S.S %	Vit.C mg/100g(f.w)	Total sugars mg/g(f.w)	T. S.S (%)	Vit.C mg/100g(f.w)	Total sugars mg/g(f.w)
Methanol	5.00 a	18.13 a	39.27 a	5.51 a	18.36 ab	39.52 ab
Bacteria	4.33 b	16.36 c	29.48 b	4.30 b	16.39 c	37.92 b
Methanol+bacteria	5.03 a	19.27 a	40.63 a	5.58 a	19.28 a	42.85 a
Control	4.26 b	17.54 b	26.91 c	4.49 b	17.72 b	27.73 c

Values in the same column followed by the same letter do not significantly differ from each other according to Duncan's multiple range tests at 5% level.

CONCLUSION

Under clay loam soil results recommend spraying squash plant grown under greenhouse in winter time with methanol plus methylotrophic bacteria as an affective agricultural strategy for increasing vegetative growth, the highest total yield and fruit quality.

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استجابة نباتات الكوسة للرش بالبكتريا الممثلة للميثانول و الميثانول تحت الزراعات المحمية هبة حنفي محمد محمد و رضا السيد أحمد حسن قسم بحوث الزراعات المحمية- معهد بحوث البساتين – مركز البحوث الزراعية - مصر

أجريت تجربتان حقليتان خلال موسمي الزراعة 2018/2017 و 2019/2018 بالصوب الزراعية في المزرعة البحثية في قها التابعة لمعهد بحوث البساتين مركز البحوث الزراعي - محافظة القليوبية. لدراسة استجابة نباتات الكوسة للرش بالبكتريا الممثلة للميثانول (10 سم³/لتر) و الميثانول (20%) وكذلك الخلط بينهم بالإضافة إلى معاملة المقارنة (الرش بالماء) وتأثير ذلك على النمو الخضري والمحصول الثمري وكذلك جودة الثمار الناتجة للصف بيرا. وصممت التجربة في قطاعات كاملة العشوائية من ثلاث مكررات. وقد أظهرت النتائج المتحصل عليها أن رش المجموع الخضري للنبات بمخلوط البكتريا الممثلة للميثانول مع كحول الميثانول ست مرات بعد الشتل بـ 20 يوم وعلى فترات كل 15 يوم أثناء موسم النمو إلى تحسن في المجموع الخضري والمحصول الثمري وصفات الجودة للثمار الناتجة. وتوصى الدراسة بأهمية رش نباتات الكوسة النامية شتاء تحت الصوب بمخلوط من الميثانول (20%) والبكتريا الممثلة للميثانول (10 سم³/لتر) لتحسين النمو الخضري وزيادة المحصول وجودته.

الكلمات الدالة: - الكوسة - البكتريا الممثلة للميثانول - كحول الميثانول- المحصول الثمري