

## Impact of Mycorrhizae and Polyethylene Mulching on Growth, Yield and Seed Oil Production of Bottle Gourd (*Lagenaria siceraria*)

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**Abstract:** The experiment was carried out in the greenhouse condition on Bottle gourd (*Lagenaria siceraria* (Mol.) Standl) during seasons of 2014/2015 and 2015/2016. The objective of this work was to investigate the effect of polyethylene mulching (black and white) and Mycorrhizae inoculation on plant growth, fruit yield, seed yield and seed oil content. Seeds of bottle gourd were sown in 15 October in the nursery and the seedlings were transplanted in greenhouse on 15 November. The experiment was designed in a split plot design in the two growing seasons. Using polyethylene mulching enhanced both of soil temperature and soil moisture content comparing to bare soil as a control. The obtained results showed that a significant increase in the vegetative growth in covered-inoculated treatments comparing with control and the black polyethylene mulch was more effective than the white mulching. For instance, the average of vine length 60 days after transplanting in the first season; in the black and white mulch without mycorrhizae treatments was 1.34 and 1.16 fold of control and with mycorrhizae, the ratio was 1.44 and 1.27 fold of control, respectively. There was a significant effect of mycorrhizae on plant growth, but the main effect of mycorrhizae treatment was more obvious in fruit characteristics in terms of size, length as well as number of seeds per fruit in both growing seasons. The benefit of this study was to obtain the high seed productivity and quality of Bottle gourd plant in addition to high seed oil production in Egypt.

**Key words:** Bottle gourd • *Lagenaria siceraria* • Polyethylene mulch • Mycorrhizae • Soil temperature • Soil moisture • Seeds production • Seeds oil

### INTRODUCTION

Bottle gourd (*Lagenaria siceraria* (Mol.) standley) is an important vegetable plant belongs to family *Cucurbitaceae* and largely cultivated in the tropics and subtropics areas. It is having a wide range of uses, such as its edible fruits, which are good source of carbohydrates and calcium, the pulp and leaves have medicinal properties [1]. In addition, its seeds represent an important source of protein and oil [2]. The bottle gourd seeds are encapsulated with innumerable phytochemicals, such as vitamins, minerals and essential amino acids along with saponin and essential fatty acids (especially, n-3) which helps to promote energy level and functional activity of brain [3]. In addition, the *L. siceraria* oil has many applications in various industries [4].

Bottle gourd gives optimum yield at sunny days and warm climate. It grows well in sandy loam soils rich in organic matter. Sandy soil and a constant temperature of 30°C is ideal for optimum seed germination (5) and germination is nil at 35°C (6). In Egypt, bottle gourd is planted primarily for the production of seeds, which can be used for eating or rarely for the production of salad oil.

The use of plastic materials for mulching is a very common practice for vegetable crops. Mulch conserves soil moisture, retained heat as well as it suppresses weed growth. Furthermore, it increases yield with higher quality, promoting faster crop development, earlier harvest and enhanced pest management [7, 8]. The most commonly used mulches are white and black. There are many advantages for using black polyethylene mulch such as increase of soil temperature, reduced weed problems,

reduce nutrient leaching and increase plant growth. Soil temperatures under black plastic mulch during the daytime are generally 3-4°F higher at a 2-in. depth and 2°F higher at a 4-in., in addition to ability to control weeds depth compared with those in bare soil [9]. White plastic mulch does not heat the soil to the level that black plastic mulch does, but it has other advantages. White plastic mulch would reflect light back to the plant canopy and enhance photosynthetic efficiency of the plant [10].

Arbuscular mycorrhizae (AM) is one of the most common symbioses worldwide and about 80 % of the known plant species form AM [11]. Mycorrhizal symbiosis play a key role in nutrient cycling in the ecosystem and protects plants against environmental and cultivation stress [12]. The effect of climatic changes in AM colonization has been studied in several plant species by many authors, most have been failed to find consistent patterns of this relationship [13]. Effect of mycorrhizal inoculation on bottle gourd plant was studied by few researchers. Varalaxmi *et al.* [14] investigated ten vegetable and fruit yielding plants belonging to Cucurbitaceae including bottle gourd for AM fungal association. They found the presence of 35 AM fungi associated with these plants and identified them up to species level. *Glomus* was represented by 17 species, *Acaulospora* by nine species, *Scutellospora* by four species, *Gigaspora* by three species and *Archaeospora* and *Entrophospora* were represented by one species. Species found to be associated to *Lagenaria vulgaris* Ser. (Bottle gourd) are *Acaulospora dilatata*, *A. laevis*, *A. nicolsonii*, *Gl. fistulosum*, *Scutellospora heterogama* and *S. tricalypta*.

In another study, Srivastava *et al.* [15] investigated bottle gourd and another 9 Cucurbitaceae species (ash gourd, water melon, ivy gourd, musk melon, cucumber, pumpkin, ridged gourd, bitter gourd and snake gourd) for Vesicular Arbuscular Mycorrhizae (VAM) fungal association. Presence of 23 VAM fungi associated with these plants was identified up to species level. Mycorrhizae playing an important role for leaching nutrition, plant physiology and plant growth alone or when interacts with other microorganisms in the root zone area and preventing rapid degradation of environment [16]. Mycorrhizal association benefits higher plants by improving plant growth against salt stress, drought stress, heavy metals and nutrient uptake storage of carbohydrates and oils [17]. *Glomus* was represented by 10 species, *Acaulospora* by six species, *Scutellospora* by three species, *Gigaspora* by three species and *Entrophospora* was represented by one species. Species

found to be associated with *Lagenaria siceraria* (Mol.) Standl (Bottle gourd) are *Glomus fasciculatum*, *Acaulospora dilatata*, *A. laevis*, *A. nicolsonii*, and *Scutellospora heterogama* and fungal structure found was arbuscules, hyphae and rhizomorph [15]. The propose of this work is to improve the growth, seeds yield and seeds oil production of bottle gourd plants as an important vegetable crop by cultivation under greenhouse conditions in winter season by using polyethylene mulches and mycorrhizae fungi inoculation treatments.

## MATERIALS AND METHODS

**Plant Materials and Cultivation:** Field experiment was conducted under greenhouse conditions at Central Laboratory for Agricultural Climate during winter seasons of 2014/2015 and 2015/2016 to investigate the effect of polyethylene mulching and Mycorrhizae inoculation on growth and oil production of bottle gourd plants. The seeds of bottle gourd (local variety) were obtained from Central Laboratory for Agriculture Climate, Agricultural Research Centre, Dokki, Egypt and sown in nursery in greenhouse on October<sup>15<sup>th</sup></sup> each season for thirty days, then transplanted to the greenhouse on November<sup>15<sup>th</sup></sup> in both seasons. The greenhouse area was 540 m<sup>2</sup> (9 m width X 60 m length) which divided to five lines and three lines of them were used for the experiment each line has 60 plants (total 60 plants /line and 180 plants /greenhouse) and each treatment consist of three replicate each replicate has 20 plants. Plants were irrigated and fertilized using the irrigation net which consists of drip lines and fertilizers supply unit. Irrigation and fertilization were applied according to recommendation of Ahmed [18].

**Mulching Treatment:** Two different polyethylene mulches (black and white) were used to cover the soil of raised beds. The thickness of polyethylene was 0.3 mm. The bare soil (without mulching) was used as control. Treatments were distributed randomly in three replicates; each replicate was 20 m in length and consisted of 20 transplants.

**Mycorrhizae Inoculation Treatment:** The mycorrhizal fungal inoculum was obtained from bio-fertilization unit, Faculty of Agriculture, Ain Shams University, Egypt. The inoculum was spores suspension and pieces of infected roots of two species i.e., *Glomus* sp. and *Gigaspora* sp. Inoculum of 10 ml containing 1000 spores and 50 g of mycorrhizal-colonized root fragments were

Table 1: The physical and chemical properties of the experimental soil

Particle size distribution (%)		Cations and anions (meq / l)	
Sand	42	Ca <sup>++</sup>	6.0
Silt	21	Mg <sup>++</sup>	3.0
Clay	37	Na <sup>+</sup>	20.1
Texture	Clay loam	K <sup>+</sup>	1.2
pH (1:2:3)	8.2	Cl <sup>-</sup>	13.0
EC (dS / m)	2.4	HCO <sub>3</sub> <sup>-</sup>	2.6
CaCO <sub>3</sub> (%)	16.0	OM (%)	0.35

placed in the planting hole during seedlings transplantation [19]. Each treatment has divided into two subplots (ten plants for each), one was treated with mycorrhizae.

**Root Staining:** For the evaluation of root colonization, roots staining technique was applied according to Koske and Gemma [20]. Root samples were dipped in water to remove adhering soil, rinsed and cut into 0.5 to 1 cm segments cleared by immersing in KOH (10%) for 45 min. at 90°C, rinsed, washed with water, acidified by immersing in HCl (1%) for 4 min and then stained with a mixture of chlorazol white E 0.03 % (CBE), lactic acid, glycerol (1:1:1), for 15 min at 90°C. Root colonization levels were estimated on 3 groups of 30 root segments randomly chosen and examined under the light microscope for presence of fungal structures.

**Spores Extraction and Count:** Spores were isolated using a mix of wet-sieving and sucrose gradient techniques [21]. 100 g of surrounding soil was rinsed in through 1000, 500, 250, 106 and 45  $\mu$ m sieves; soil was recovered from 45  $\mu$ m sieve, suspended in water and centrifuged at 3000 rpm for 3 min. The supernatant was discarded and the soil sediment was resuspended in a sucrose solution (60%) and centrifuged at 1000 rpm for 2 min. The supernatant containing spores was filtered under vacuum on filter

paper (Whatman # 1). The spores were counted using the light microscope and the estimated number of spores was attributed to 100 g of soil.

**Soil Physical and Chemical Analysis:** The experimental soil was analyzed two weeks before work. The physical and chemical properties of the experimental soil are shown in Table (1).

**Climatic Data:** Climatic data, maximum (T. max. °C) and minimum (T. min. °C) air temperature and relative humidity (RH %) were recorded under greenhouse conditions by the meteorological station of CLAC during both growing seasons and the data are shown in Table (2).

**Oil Extraction:** The effect of different treatments on seeds oil content was estimated by extracting oils from seeds using Soxhlet extractor as follow: Seed were grinded by batch mill (IKA, M20) using sieve 0.2mm. The oil contents of seeds were determined by complete extraction. 50 g of the grounded shelled seed was packed into a weighed thimble, which was introduced into the soxhlet system. The extraction process was carried out six hours and the temperature was corresponded to the boiling point of the solvent used. Solvent used was n-hexan (65°C) which was obtained from El Nasr Company for Chemicals, Cairo, Egypt. Solution obtained of the extracted oil was stripped of the remnant solvent using vacuum distillation by rotary evaporator (Heidolph, laborota 4000) at a same temperature. The oil obtained was weighed and the percentage oil content was calculated [22].

**Soil Temperature and Soil Moisture Content:** Soil temperature (°C) was estimated regularly every week at 20 cm depth of soil by using soil thermometer during the both growing seasons under different treatments of polyethylene mulching. Besides, the soil moisture (%) was

Table 2: Average maximum and minimum air temperature and relative humidity under greenhouse condition during both growing seasons

Month	First season (2014-2015)			Second season (2015-2016)		
	T. max. °C	T. min °C	RH (%)	T. max. °C	T. min °C	RH (%)
Nov.	25.7	13.9	65.0	25.7	16.4	65.9
Dec.	23.3	10.9	67.9	20.6	10.7	68.2
Jan.	21.4	8.9	72.0	19.9	8.2	57.8
Feb.	22.6	10.4	67.4	20.9	9.0	56.4
Mar.	25.4	13.2	55.2	21.0	10.2	56.1
Apr.	29.6	16.1	49.2	25.6	14.0	48.9
May	32.8	19.4	46.7	32.5	19.4	48.1
June	34.5	21.1	44.9	34.2	21.1	46.3
July	35.2	21.5	43.1	34.9	21.6	44.5

estimated under different covers of polyethylene in the both growing seasons, as well by using soil moisture meter.

**Plant Growth Measurements:** For the evaluation of plant growth, sample of three plants from each sub plot were taken randomly after 60 days from transplanting to record some growth parameters, i.e., vine length (cm), number of leaves, number of branches, sex ratio, leaves fresh and dry weight (g), vine fresh and dry weight (g) and plant fresh and dry weight (g), for dry weight, plant samples were dried at 70 °C in the oven for 72 hours. In addition, the leaf area (cm<sup>2</sup>) was calculated for the fourth leaf from the growing tip according to Shaik and Murthy [23] by this equation  $y = -58.66 + (19.77 \times L)$  where y is the leaf area cm<sup>2</sup> and L is the leaf length.

**Chemical Analysis:** For the chemical composition estimation, sample of the fourth top leave from the apical tip of plant was dried at 70 °C for 72 hours. Then it was digested to determine N % P % and K % contents. Total nitrogen (%) in leaves was determined by using the micro kjeldahl according to A.O.A.C. [24]. Phosphorus (%) was determined calorimetrically at 550 nm as described by.....?? and Potassium (%) was determined by flame photometer as described by Ranganna [25].

**Yield and its Components:** The ripened and dry fruits were harvested at the end of the growing season and the seeds were removed, cleaned, dried in the open air and stored until processing. For the evaluation of fruit and seed characteristics, the number of fruits / plant, fruit length (cm) fruit diameter (cm), the number of seeds / fruit, seeds weight (g / fruit), seeds yield (g / plant) and seeds yield (kg / greenhouse).

**Statistical Analysis:** All obtained data were subjected to statistical analysis for variance by using split plot design as mentioned by Gomez and Gomez [26] for calculating the least significant differences (LSD) between treatments. In addition, Duncan test was used to comparing between means.

## RESULTS AND DISCUSSION

**Soil Temperature:** Data in Fig. 1 shows the effect of different polyethylene mulching on soil temperature during both growing seasons. Obviously, both of polyethylene mulches such as black and white had a positive effect on soil temperature (°C) values comparing to control treatment (bare soil). The highest values of soil temperature (°C) were obtained by using black polyethylene mulch followed by using the white one, while the lowest values of soil temperature were obtained by using bare soil treatment in both growing seasons. These results may be due to that using polyethylene mulching decrease the soil heat flux comparing with bare soil. Besides, black mulch promotes a relatively large net radiation at the soil surface. These results are in agreement with those obtained by..... [8] and .....[27].

**Soil Moisture Content:** Data presented in Fig. 2 displayed the effect of different polyethylene mulches on soil moisture content (%) during both growing seasons. It was clearly noted that both of polyethylene mulching had a significant effect on soil moisture content (%) compared to bare soil treatment. The highest values of soil moisture content (%) were obtained by using black polyethylene mulch followed by using white mulch. The lowest values of soil moisture were obtained by using bare soil (control) treatment in the both growing seasons. These results may

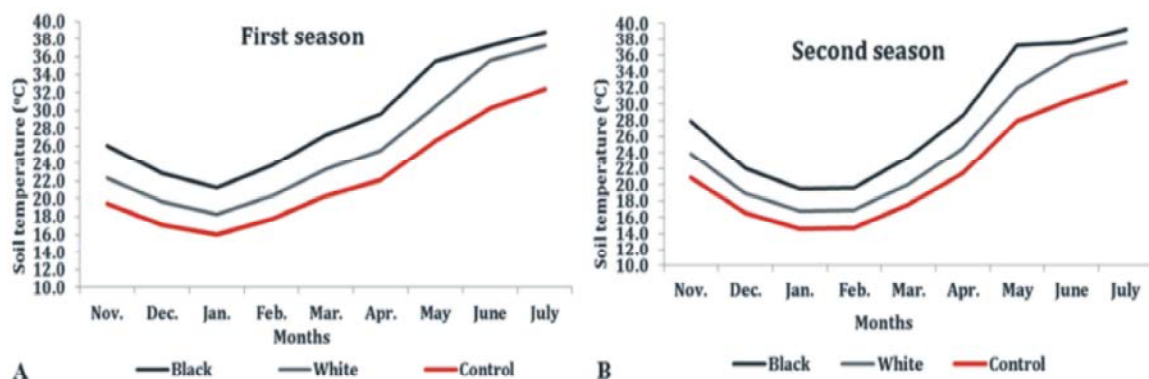


Fig. 1: The effect of different polyethylene mulches on soil temperature during the first season (A) and second season (B).

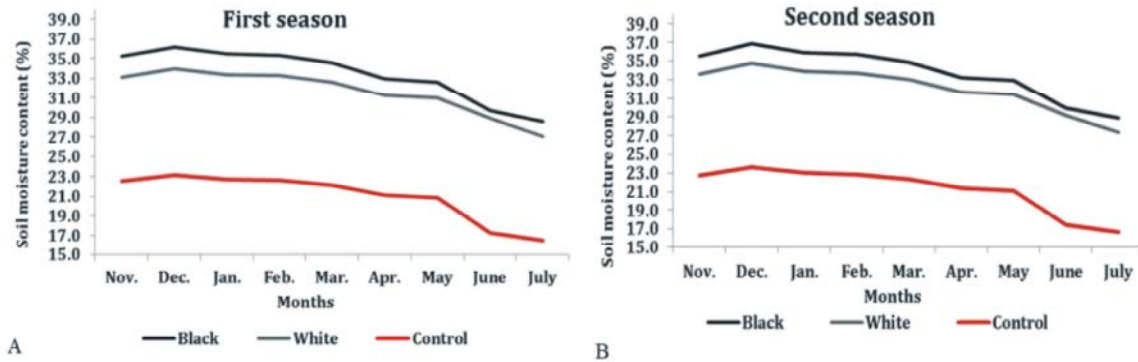


Fig. 2: The effect of different polyethylene mulches on soil moisture content during the first season (A) and second season (B).

Table 3: Effect of polyethylene mulching, Mycorrhizae and their interaction on vine length (cm), number of leaves, number of branches and leaf area (cm<sup>2</sup>) of bottle gourd plant during the two seasons.

	MC	M	Vine length (cm)		No. of leaves / plant		No. of branches / plant		Leaf area (cm <sup>2</sup> )	
			1 <sup>st</sup> season	2 <sup>nd</sup> season	1 <sup>st</sup> season	2 <sup>nd</sup> season	1 <sup>st</sup> season	2 <sup>nd</sup> season	1 <sup>st</sup> season	2 <sup>nd</sup> season
Specific effect	C		259.7C	264.9C	51.8C	52.4C	2.7 B	2.7C	255.7C	254.3C
	B		348.0A	355.0A	137.8A	139.2A	4.8A	5.9A	344.2A	352.9A
	W		303.8B	309.9B	97.2B	98.1B	4.5A	4.5B	299.4B	307.8B
		M0	292.2B	298.1B	89.3B	90.2B	4.3A	4.4A	282.3B	281.8B
		M1	315.4A	321.8A	101.9A	102.9A	4.3A	4.4A	317.3A	328.2A
Interaction effect	C	M0	250.0f	255.0f	47.7f	48.1f	2.7c	2.7c	240.2e	225.2e
		M1	269.3e	274.7e	56.0e	56.6e	2.7c	2.7c	271.3d	283.5d
	B	M0	336.0b	342.7b	133.3b	134.7b	5.7a	5.7a	319.2b	325.8b
		M1	360.0a	367.2a	142.3a	143.8a	6.0a	6.1a	369.2a	380.0a
	W	M0	290.7d	296.5d	87.0d	87.9d	4.7b	4.7b	287.5c	294.5c
		M1	317.0c	323.3c	107.3c	108.4c	4.3b	4.4b	311.4b	321.1b

Values of specific or interaction effect followed by the same capital and small letters are not significantly different based on Duncan's Test at 5% level. Mulch colors (MC), white (W), Black (B), bare soil without mulch called control (C), Mycorrhizae (M), untreated with Mycorrhizae (M0), treated with Mycorrhizae (M1)

be due to the role of polyethylene mulching in reducing the evaporation rate from soil surface and led to increase the soil moisture content. These results are in agreement with those obtained by.... [7],.....[ 8] and .....[28].

**Vegetative Growth:** Data in Table 3 show the effect of polyethylene mulching, mycorrhizae inoculation and their interaction on the vegetative growth characteristics such as vine length (cm), number of leaves per plant, number of branches per plant and leaf area (cm<sup>2</sup>). Regarding to the effect of polyethylene mulching, it was clearly noticed that vegetative growth characteristics increased significantly with using white and black mulch comparing to the bare soil (control) during both experimental seasons. The highest value of mentioned characteristics was obtained from black mulch during both seasons. The specific effect of mycorrhizae was cleared in case of vine length, number of leaves per plant and leaf area, but was not significant in case of number of branches per plant

during both seasons. Concerning to the interaction between mulching treatment and mycorrhizae showed that both treatments enhanced vegetative characteristics comparing to control such as bare soil with mycorrhizae (CM1) or bare soil without mycorrhizae (CM0). Black mulch plus mycorrhizae (BM1) gave the highest value of plant vine length (269.3 and 367.2 cm), highest number of leaves / plant (142.3 and 143.8), number of branches / plant (6 and 6.1) and leaf area (369.2 and 380 cm<sup>2</sup>) compared to the control (CM0) in the 1<sup>st</sup> and 2<sup>nd</sup> season, respectively.

The exhibited results in Table 4 displayed the effect of polyethylene mulching and mycorrhizae treatments on the total fresh and dry weight of plant (g), results were in corresponding with presented results in Table 3. The specific effect of mulching and mycorrhizae treatments had significant affect on fresh and dry weights values compared with control in the both growing seasons. Regarding to the interaction effects, it was

Table 4: Effect of polyethylene mulching, Mycorrhizae and their interaction on leaves fresh weight (g), vine fresh weight (g) and plant fresh weight (g) of bottle gourd plant during the first and second seasons.

	MC	M	Plant fresh weight (g)		Plant dry weight (g)	
			1 <sup>st</sup> season	2 <sup>nd</sup> season	1 <sup>st</sup> season	2 <sup>nd</sup> season
Specific effect	C		333.3C	374.8C	64.1C	64.7C
	B		1241.8A	1394.5A	179.3A	181.1A
	W		774.8B	871.2B	101.7B	102.7B
		M0	708.1B	794.9B	107.6B	108.7B
		M1	858.5A	965.5A	122.4A	123.6A
Interaction effect	C	M0	330.5f	372.3f	60.8f	61.4f
		M1	336.0e	377.4e	67.3e	68.0e
	B	M0	1123.0b	1258.2b	174.7b	176.5b
		M1	1360.7a	1530.9a	183.8a	185.7a
	W	M0	670.7d	754.2d	87.3d	88.2d
		M1	879.0c	988.2c	116.0c	117.2c

Values of specific or interaction effect followed by the same capital and small letters are not significantly different based on Duncan's Test at 5% level. Mulch colors (MC), white (W), Black (B), bare soil without mulch called control (C), Mycorrhizae (M), untreated with Mycorrhizae (M0), treated with Mycorrhizae (M1)

Table 5: Effect of polyethylene mulching, Mycorrhizae and their interaction on sex ratio, number of fruits / plant, fruit length (cm), fruit diameter (cm) and number of seeds / fruit of bottle gourd plant during the first and second seasons

	MC	M	Sex ratio (male / female)		No. of fruits /plant		Fruit length (cm)		Fruit diameter (cm)	
			1 <sup>st</sup> season	2 <sup>nd</sup> season	1 <sup>st</sup> season	2 <sup>nd</sup> season	1 <sup>st</sup> season	2 <sup>nd</sup> season	1 <sup>st</sup> season	2 <sup>nd</sup> season
Specific effect	C		4.4A	4.5A	1.7C	1.7C	49.0C	50.0C	8.1C	8.2B
	B		2.7C	2.7C	4.1A	4.1A	68.2A	69.5A	10.9B	11.0A
	W		3.7B	3.8B	3.4B	3.4B	64.0B	65.3B	11.0A	11.1A
		M0	3.6A	3.7A	2.9B	2.9B	53.9B	55.0B	9.6B	9.7B
		M1	3.6A	3.7A	3.2A	3.3A	66.9A	68.2A	10.4A	10.5A
Interaction effect	C	M0	4.5a	4.6a	1.6f	1.6f	44.0f	44.9f	7.7d	7.8b
		M1	4.4b	4.5b	1.7e	1.8e	54.0e	55.1e	8.5c	8.6b
	B	M0	2.7e	2.7e	3.9b	4.0b	59.7c	60.9c	10.5b	10.6a
		M1	2.7e	2.7e	4.2a	4.3a	76.7a	78.2a	11.2a	11.3a
	W	M0	3.6d	3.7d	3.1d	3.1d	58.0d	59.2d	10.7b	10.8a
		M1	3.8c	3.8c	3.7c	3.7c	70.0b	71.4b	11.4a	11.5a

Values of specific or interaction effect followed by the same capital and small letters are not significantly different based on Duncan's Test at 5% level. Mulch colors (MC), white (W), Black (B), bare soil without mulch called control (C), Mycorrhizae (M), untreated with Mycorrhizae (M0), treated with Mycorrhizae (M1)

found that the high increase in fresh and dry weight was basically due to the effect of mulching more than to mycorrhizae. In this regard, black polyethylene mulch was notably better than white mulch even with or without mycorrhizae treatment and gave the highest values of plant fresh and dry weight in both growing seasons. Using white mulch and mycorrhizae (WM1) treatment came in the second rank. The worst results for fresh and dry weight of plant obtained from cultivated in bare soil without mycorrhizae inoculation in both studied seasons. These results agreed with what have been mentioned before about benefits of using mulches and are in agreement also with those reported by Parmar *et al.* [30], who mentioned that bottle gourd is a large plant, it require more water, using mulch is reducing soil water loss which

maintain more uniform soil moisture and reduce irrigation frequency. Fertilizers are not lost by leaching underneath mulches, so that the used fertilizers are not wasted. The root and vegetative of bottle gourd was enhanced when grown under mulch, because microbial activity, adequate oxygen, soil under plastic mulch remains loose, friable and well ventilate [31].

**Flowering and Fruit Characteristics:** Results which related to flowering action and ripped fruits characteristics are shown in Table 5. Regarding to sex ratio (male flowers / female flowers) it was significantly low in black mulch treatment in comparison with other mulching treatment that means black mulch encouraged the initiation of female flowers. Besides, there was no significant effect of

Table 6: Effect of polyethylene mulching, Mycorrhizae and their interaction on seeds weight (g / fruit), seeds yield (g / plant) and seeds yield (Kg /Greenhouse) of bottle gourd plant during the first and second seasons.

	MC	M	No. of seeds / fruit		Seeds weight (g/ fruit)		Seeds yield ( g / plant)		Seeds yield (kg) /Greenhouse	
			1 <sup>st</sup> season	2 <sup>nd</sup> season	1 <sup>st</sup> season	2 <sup>nd</sup> season	1 <sup>st</sup> season	2 <sup>nd</sup> season	1 <sup>st</sup> season	2 <sup>nd</sup> season
Specific effect	C		382.3C	386.2C	72.4C	73.1C	123.1C	124.3C	36.9C	37.3C
	B		616.3A	622.5A	176.1A	177.8A	722.0A	729.A	216.6A	218.7A
	W		599.2B	605.2B	150.5B	152.0B	511.7B	516.8B	153.5B	155.0B
		M0	467.1B	471.8B	119.9B	121.1B	347.7A	351.2B	104.3B	105.4B
		M1	598.1A	604.1A	146.1A	147.6A	467.5B	487.1A	140.3A	146.1A
Interaction effect	C	M0	317.3f	320.5f	68.1f	68.8f	109.0e	110.1e	32.7e	33.0e
		M1	447.3e	451.8e	76.7e	77.5e	130.4d	139.5d	39.1d	41.9d
	B	M0	554.0c	559.5c	154.9c	156.4c	604.1b	625.6b	181.2b	187.7b
		M1	678.7a	685.5a	197.3a	199.3a	828.7a	857.0a	248.6a	257.1a
	W	M0	530.0d	535.3d	136.7d	138.1d	423.8c	428.1c	127.1c	128.4c
		M1	668.3b	675.0b	164.4b	166.0b	608.3b	614.2b	182.5b	184.3b

Values of specific or interaction effect followed by the same capital and small letters are not significantly different based on Duncan's Test at 5% level. Mulch colors (MC), white (W), Black (B), bare soil without mulch called control (C), Mycorrhizae (M), untreated with Mycorrhizae (M0), treated with Mycorrhizae (M1)

mycorrhizae treatment, which means mycorrhizae had no effect in flowering process which contradicts with Pendleton [32] where his results suggest that mycorrhizal colonization may differentially influence male and female components of plant reproduction. In contrast, mycorrhizae had high effect on the other measurements, which reflects in the highest number of fruits per plant and fruit characteristics such as fruit length and diameter particularly when combined with the effect of black mulch. Concerning the interaction, using mycorrhizae and black mulch gave the highest values of fruit characteristics in both tested seasons. Generally mycorrhizal inoculation results were not as expected, may that agreed with Xiang-xi *et al.* [33], where they investigated mycorrhizal dependence (MD) of five vegetables in cucurbitaceae to two arbuscular mycorrhizal fungi *Glomus mosseae* (G.M.) and *Gigaspora rosea* (Gi.r) under greenhouse conditions. Results showed that the growth, leaf area, photosynthetic rate and dry mass were significantly enhanced with the order of MD of the vegetables being cucumber > watermelon > balsam pear > bottle gourd > squash.

**Seeds Yield and its Components:** Seed is the most important part of bottle gourd plant in this study because its content of edible oil. The obtained results in Table 6 refers to values of some seeds measurements such as number of seeds per fruit, seeds weight (g / fruit), seeds yield (g / plant) and seeds yield (Kg / Greenhouse). Generally, results are consistent with previous results. The black mulch gave the highest significant values among mulching treatments with register number of seeds / fruit (616.3 and 622.5), seed weight g/fruit (176.1 and 177.8 g), seed yield / plant (722.1 and 729 g) and seed yield

/ greenhouse (216.6 and 218.7 kg) compared to the control treatment which share the least significant level in both seasons, respectively. Polyethylene mulch produce larger fruit and have higher fruit yield per plant because of the better plant growth due to favorable hydrothermal regime of soil and complete weed free environment [9, 34-38].

Treating plants with mycorrhizae also gave significant results in all mentioned seeds characteristics compared to untreated plants in both seasons. Concerning to the interaction between mulching and mycorrhizae recorded very high values compared with untreated plants. Black mulch plus mycorrhizae (BM1) gave the highest value of seeds number / fruit (678.7 and 685.5), seed weight g/fruit (197.3 and 199.3 g), seed yield / plant 828.7 and 857 g) and seed yield / greenhouse (248.6 and 257.1 kg) compared to the control treatment which share the least significant level in both seasons, respectively. Arbuscular mycorrhizal fungi increase the absorption of food elements from soil which increase the vegetable yields [39]. The mechanisms of mycorrhizae was involved in interactions with organics include direct effects of enzymes secreted by hyphae and indirect effects of enhanced root-derived enzymes, microbial activity and modified microbial composition to increase nitrogen capture from complex organic material and increased in the presence of the organic material [40-43].

For recognizing the effect of mulch and mycorrhizae treatments in the analytic level, percentage of nitrogen, phosphorus and potassium in leaves were measured in addition to the percentage of oil in seeds. Table 7 shows a significant increase on NPK percentage in white and black treatments in comparison with control, indicating that effect of mulch not only helping in increasing plant

Table 7: Effect of polyethylene mulching, Mycorrhizae and their interaction on N, P and K content (%) of leaves and oil content (%) of seeds of bottle gourd plant during the first and second seasons

			Leaves Mineral Content							
			N %		P %		K %		Oil Percentage (%)	
	MC	M	1 <sup>st</sup> season	2 <sup>nd</sup> season	1 <sup>st</sup> season	2 <sup>nd</sup> season	1 <sup>st</sup> season	2 <sup>nd</sup> season	1 <sup>st</sup> season	2 <sup>nd</sup> season
Specific effect	C		3.1C	3.1C	3.39C	3.42C	0.310C	0.316C	21.3C	21.9C
	B		3.9A	4.0A	4.37A	4.42A	0.438A	0.447A	26.7A	25.4A
	W		3.6B	3.6B	3.98B	4.02B	0.382B	0.389B	23.7B	23.8B
		M0	3.4B	3.5B	3.77B	3.80B	0.354B	0.362B	23.5B	24.0B
		M1	3.6A	3.7A	4.06A	4.10A	0.399A	0.407A	24.2A	24.5A
Interaction effect	C	M0	2.9f	2.9e	3.24f	3.27f	0.290e	0.296e	21.1f	22.2f
		M1	3.3e	3.3d	3.54e	3.57e	0.330d	0.337d	21.5e	21.6e
	B	M0	3.9b	3.9b	4.16b	4.20b	0.410b	0.418b	26.2b	26.5b
		M1	4.0a	4.0a	4.58a	4.63a	0.467a	0.476a	27.1a	27.5a
	W	M0	3.6d	3.6c	3.90d	3.94d	0.363c	0.371c	23.3d	23.2d
		M1	3.7c	3.7c	4.07c	4.11c	0.400b	0.408b	24.1c	24.3c

Values of specific or interaction effect followed by the same capital and small letters are not significantly different based on Duncan's Test at 5% level. Mulch colors (MC), white (W), Black (B), bare soil without mulch called control (C), Mycorrhizae (M), untreated with Mycorrhizae (M0), treated with Mycorrhizae (M1)

vegetable growth but also increasing its contents of chemical elements, which was reflected on the contents of seeds of oil. Specific effect of white and black mulch treatments on seed oil contents was remarkable, where the oil percentage means were 26.7 in the first season and 25.4 in the second season for black mulch treatment and were 23.7 in the first season and 23.8 in the second season for white mulch treatment, while control gave 21.3 in the first season and 21.9 in the second season.

The obtained data from effect of mycorrhizae on NPK and seed oil content showed the same trend as indicated in previous Tables (3, 4 and 6). The highest amount of NPK and oil content percentage was significantly increased from treatment with mycorrhizae compared with the untreated plants. Application of arbuscular mycorrhiza fungi increased water relations, nutrient uptake and perform as bio-protectants against toxic stresses and pathogens. In order to further improve their benefits, it is necessary to ensure the management practices comprising low tillage, stop abundance of using chemical fertilization and reduce the used phosphorus fertilization [44-46].

Results of interaction effects on NPK and seed oil percentage show that BM0, BM1, WM0 and WM1 gave close results, but significantly higher than CM0 and CM1. The highest values in percentage of nitrogen (4 and 4 %), phosphorus (4.58 and 4.63 %), potassium (0.467 and 0.476 %) and seed oil percentage (27.1 and 27.5 %) were obtained from black mulch plus mycorrhizae treatment (BM1) compared with untreated plants during both seasons, respectively. These results indicated that the major effect due to the effect of mulch. Mulch increased

growth and fruit yield of tomato through modification of the crop growing environment by reducing weed infestation, soil moisture depletion and ameliorating soil temperatures [47]. Mulch could improve leaf photosynthetic capacity beside the role of polyethylene for enhancing root growth, as well as, absorption of each of water and nutrients and thereby, enhanced metabolic activities within plant during the period of growth and reproduction process, which possessed much shoot number per plant and width leaf area with high leaf chlorophyll content as mentioned before that induced more photosynthetic rates [9, 48, 49].

**Root Staining:** Roots of bottle gourd plant were focused for mycorrhizal colonization using chlorazol white E 0.03 % according to Koske and Gemma [20]. Fungal structures found were arbuscules and hyphae this fungal structure found *Lagenaria vulgaris* Ser. Srivastava *et al.* [15] was arbuscules, hyphae and rhizomorph.

**Root Infection and Spores Count:** To study the interaction between bottle gourd plants and mycorrhizae, percentage of infection in root system (RS) and spores count (SC) in rhizosphere were estimated. The obtained data shows that there was low natural infection when no mycorrhizal inoculation was used in CM0, BM0 and WM0 treatments. In the mycorrhizal treated treatments (BM1, WM1 and Table (8): The effect of plastic mulches and mycorrhizae on N, P, K contents (%) in leaves and seeds oil percentage (%) of bottle gourd plant during 2015 and 2016 growing seasons. CM1), the RS and SC were notable



Table 8: Number of spores and percentage of root infection in different treatments

Treatment	Season	Root infection (%)	Spores count / 100 (g) soil
CM0	1 <sup>st</sup>	1	6
	2 <sup>nd</sup>	0	11
BM0	1 <sup>st</sup>	2	17
	2 <sup>nd</sup>	3	19
WM0	1 <sup>st</sup>	1	32
	2 <sup>nd</sup>	0	15
CM1	1 <sup>st</sup>	12	78
	2 <sup>nd</sup>	21	117
BM1	1 <sup>st</sup>	20	146
	2 <sup>nd</sup>	25	255
WM1	1 <sup>st</sup>	22	206
	2 <sup>nd</sup>	30	319

C = Control, B = Black, W =White, Mycorrhizae (M), M0 = untreated, M1 = Treated

higher. Also, it was noted that using mulches increased mycorrhizal infection, where results of BM1 and TM1 were higher than results of CM1, (Table 8). The highest RS and SC was recorded for TM1 (30 % and 319/100g soil); this result is different from result obtained by that Srivastava *et al.* [15], when they used different species of mycorrhizae, where found that PI was 43 % and SC was 1900 / kg.

## CONCLUSION

The effect of black polyethylene mulch when combined with mycorrhizae inoculation gave clearly positive effect on vegetative growth and chemical constituents characteristics of bottle gourd plants, in addition, enhanced the seeds yield and their oil content. This study may bull attraction to bottle gourd plants as an important vegetable crop could be participate in fulfilling the world nutritional needs and reduces the gap between oil production and oil demand in Egypt. The efficacy of these treatments increased plant yield and nutrient uptake and seed oil production.

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