Archives of Agriculture Sciences Journal
Print ISSN: 2535-1680
Online ISSN: 2535-1699

ARCHIVES OF AGRICULTURE SCIENCES JOURNAL

Volume 6, Issue 2, 2023, Pages 191–205

Available online at www.agricuta.edu.eg

DOI: https://dx.doi.org/10.21608/aasj.2023.318552

Improving the productivity of khella (*Ammi visnaga* L.) plants by using bio-straw treatments and some microorganisms

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Abstract

A field experiment was conducted during the two successive seasons of 2021/2022 and 2022/2023 at the Experimental Farm, Faculty of Agriculture, Al-Azhar University, Assiut, Egypt to examine the effect of bio straw (spent mushroom compost "SMC") at 0, 15, 20 and 25 m^3/ feddan (= 4200m^2) half of the recommended doses of NPK fertilizer and Microorganisms inoculation (*Streptomyces rochei* [MA-6] and *Bacillus subtilis* [MA-13] alone or in combination, as well as, their interactions on plant growth, yield characteristics and some active constituents of khella (*Ammi visnaga* L.) plants. The obtained results showed that the use of bio-straw at all levels and NPK_{HR} led to a significant increase in plant height, number of branches/ plant, weight of fresh and dry herbs in g/ plant, number of umbels/ plant, fruit yield in g/ plant and kg/ feddan and the percentages of khellin, visnagin and total chromones of seed khella (*Ammi visnaga* L.) plants. The application of a high level of bio-straw (25 m^3/ feddan) gave the highest values for these above traits. Apparently, application of microorganisms' inoculation by MA-6 and MA-13 resulted a significant increase in the above parameters. The highest values of these parameters were detected by inoculation with two strains together during the two seasons. The interaction effect on all studied variables was statistically significant and it is clear that the use of most combined treatments led to a significant increase in these all parameters. Generally, the addition of the high rate of bio-straw plus inoculation mixed of two strains was the most effective treatment in increasing these parameters.

Keywords: khella, microbial inoculants, *Streptomyces rochei*, *Bacillus subtilis*, khellin, visnagin, total chromones.

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1. Introduction

Khella (*Ammi visnaga*, L.) is one of the important pharmaceutical plants belonging to Family Apiaceae (Umbelliferae). Khella is a medicinal plant native to the Mediterranean region, where it has been used in the treatment of angina and other heart ailments since the time of the pharaohs. The plant is widely cultivated in Egypt and the Mediterranean region and the Near East. It is a winter annual herb attaining a height of about one to two meters. The fruits contain a crystalline bitter principles khellin, visnagin and khellol glucoside, in addition, about 12% proteins and up to 18 % fixed oil. Khellin and visnagin are the active ingredients of many used drugs as diuretic and antispasmodic in case of urethral stones. In recent years chloroform extract of *Ammi visnaga* seeds is used in against *Sitophilus oryzae* infesting wheat grains. In Egypt, the environmental conditions are most suitable for the cultivation and production of *Ammi visnaga*, L. (Khalil *et al*., 2020). Seeds *of Ammi visnaga* contains about 1% of khellin, which is the most important active crystalline constituent and has been synthesized and is accompanied by the two other crystalline compounds, visnagin (about 0.1%) and khellol glycoside (about 0.3%). The drug of the dried ripe fruits and it has long been used in Egypt, Khellin, which is now commercially available in tablets and injection, is a potentcoronary vasodilator. Adecoction is used to ease the passage of kidney calculi (Evans, 1998). Also, essential oil of *Ammi visnaga* inhibited a wide range of fungi and affected as antibacterial substance. Fertilizers and additives have become a way of plant life, creating their nutrient request. But from the point of environmental view, it is necessary to demonstrate the risks of mineral fertilizers and find out new possibilities for a good sensorial aspect of other eco- friendly amendments. Consequently, there is a demand for clean and safe natural biofertilizers and this could apply by using biofertilizers of microbial origin. Synthetic fertilizers, especially nitrogen, can seriously deplete the nutritional content of foods. Nitrate, the final breakdown product of nitrogen fertilizers, accumulates in ground water due to steep increase using mineral nitrogen and thus can be severely affect human health. N_2-fixing cyanobacteria, blue-green algae (BGA) have potential applications in agriculture areas, as nutrient supplements, as biofertilizer, plant growth promoting and as biocontrol agents (Gupta *et al*., 2013). Can be used as an alternative to synthetic nitrogen and make a major contribution to the soil fertility and assist crops by supplying plant growth substances (Shariatmadari *et al*., 2011). The application of organic fertilizer is preferable to mineral fertilization for improving the quality of crops, especially medicinal and aromatic plants, and organic farming has become a quality standard that small farmers in Egypt can match (Abou El-Fadl *et al*., 1990). The use of organic fertilization is more acceptable than the use of inorganic fertilizers and small farmers in Egypt could effectively match the high standards of organic farming. Several researchers have found that organic fertilization increases herb dry weight, umbels number, seed yield and some active constituents. These investigators also provided by Mahmoud (2016) on khella plants, Khalid and Shafei (2005) on dill plant, Hassan (2005) on guar and fenugreek plants, Azzaz *et al*. (2009) on fennel, Shehata *et al*. (2011) on snap bean, Rekaby (2013) on coriander, Hassan (2005) on guar and fenugreek plants. *Bacillus subtilis* is a common plant growth promoting rhizobacteria (PGPR) in soil that plays a key role in conferring biotic and abiotic stress tolerance to plants by induced systemic resistance (ISR), biofilm formation and lipopeptide production. As a part of bioremediating technologies, *Bacillus* spp. can purify metal contaminated soil. It acts as a potent denitrifying agent in agro ecosystems while improving the carbon sequestration process when applied in a regulated concentration. Although, it harbours several antibiotic resistance genes (ARGs), it can reduce the horizontal transfer of ARGs during manure composting by modifying the genetic makeup of existing microbiota. Soil texture, type, pH and bacterial concentration play a crucial role in the regulation of all these processes. The complex plant-microbe interactions could be decoded using transcriptomics, proteomics, metabolomics which would be beneficial for both crop productivity and the well-being of soil microbiota. Plant growth is enhanced by PGPR through the induction of systemic resistance, antibiosis and competitive omission. Thus, the application of microbes can be used to induce systemic resistance in plants against biotic agents and enhance environmental stress tolerance (Hashem *et al*., 2019; Mahapatra *et al.*, 2022). Actinomycetes are gram positive, aerobic and mycelia-forming bacteria, known for nutrient cycling, production of secondary metabolites and plant growth promotion (PGP) ability. A ctinomycetes are found in various habitats including sea water, fresh water, soil, marsh area etc., however, they are dominant in dry, humic and calcareous type of soil. Actinomycetes, also play an important role in the rhizosphere by secreting a wide range of antimicrobial products, thus, preventing growth of common root pathogens. However, apart from their biocontrol potential, other PGP traits of Actinomycetes are scarcely reported. There are few reports regarding their ability on phosphate solubilization, organic acid production, siderophore production and secretion of large number of enzymes, which directly or indirectly help plant growth (Al-Aksar, 2012; Doumbou *et al.*, 2001; Sadeghi *et al.*, 2012). *Streptomyces rochei* proved to possess a high capacity for the production of auxins, gibberellins and cytokinin-like substances, together with substantial levels of α-amylase and proteinase (Aldesuquy *et al.*, 1998; Jog *et al.*, 2012).

This field experiment was conducted to evaluate the use of bio-straw levels and half the recommended dose of mineral fertilizer, as well as some microbial inoculants on the growth, yield and active ingredients of khella plants.

2. Materials and methods

2.1 Experimental site and treatments description

This experiment was conducted at the Experimental Farm, Faculty of Agriculture, Al-Azhar University, Assiut, Egypt, throughout the two successive seasons of 2021/2022 and 2022/2023 in order to improve the growth and production of khella plants. The experiment included two factors: the first was rates of bio-straw and half the recommended dose of mineral fertilizer and the second was two types of microbial inoculants, as well as their interactions. The Experimental design of this study was a split-plot design with three replicates, using a randomized complete blocks design (RCBD), bio-straw levels and half the recommended dose of mineral fertilizer (five treatments) were the main plot and types of microbial inoculants (four treatments) considered the sub-plots. Therefore, the interaction treatments were 20 treatments. Khella seeds were obtained from the Agricultural Research Centre of the Department of Medicinal and Aromatic Plants in Dokky, Giza, Egypt. On November 5th, during the two seasons, fruits were immediately sown in the plot 3×4 meters. Each experimental unit had five rows that were each 3 meters long, 60 cm separated the ridges, while 40 cm apart in the hills. Each hill received about 5-6 fruits, after 40 days from sowing date, the plants were thinned to 2 plants/hill, as a result, the experimental unit included 100 plants. The physical and chemical properties of the experimental farm soil are tabulated in Table (1) as reported by Chapman and Pratt (1975).

Table (1): Physical and chemical properties of the experimental soil.

Soil character	Values	Soil character	Values
Soil texture	Clay loam	Total N%	0.12
Sand (%)	25.30	Available P (ppm)	0.14
Silt (%)	39.40	Available K (mg/100 g soil)	3.5
Clay (%)	35.30	Soluble Ions (meq/L)	
Organic matter (%)	0.57	Ca	3.4
Caco3 (%)	2.53	Mg	1.9
PH (1:2.5)	7.5	Cl	2.2
E.C (m.mohs/cm)	2.2	So4	6.6
		Co_3 + Hco_3	2.9
		Ca	3.4

2.2 Rates and method of adding bio-straw and microbial inoculants

Bio-straw levels at 0, 15, 20 and 25 m^3/feddan were added during the preparation of soil to the cultivation, in the two seasons. Physical and chemical properties of the applied bio straw were shown in Table (2). Ammonium sulphate (20.5% N), calcium superphosphate (15.5% P_2O_5), and potassium sulphate (48.5% K_2O) were the three NPK rates that were the half recommended does: 100, 100 and 50 kg/feddan, respectively. During soil preparation, phosphorus fertilizer was added in its entirety. Whereas fertilizers for nitrogen and potassium were applied at two batches, after 45 days from planting date at one-month intervals thereafter.

Table (2): The physical and chemical properties of the used bio-straw.

Properties	Value	Properties	Value
Dry weight of 1 m3	430 kg	Total N (%)	2.72
Moisture (%)	25-30	Total P (%)	0.92
pH (1:2.5)	7.84	Total K (%)	2.88
EC(dSm^{-1})	4.17	Fe (ppm)	124
Organic matter %	54	Mn (ppm)	85
Organic carbon %	31.4	Zn (ppm)	32
C/N ratio	13.79		

2.3 Microorganism strains

Both local *Streptomyces rochei* (MA-6) and *Bacillus subtilis* (MA-13) microbial strains were kindly provided by Microbiology Department, Soils, Water and Environment, Research Institute (ARC), Giza, Egypt where both strains were isolated, purified and identified as mentioned by Ibrahim *et al.* (2020). The biofertilization with bacterial strains developed on plant-based culture media temperature 30 °C for 24-48 hours .The amounts of addition to both strains, either individually or together, were 20 liters per feddan with irrigation in three times, the first after 30 days, the second after 45 and the third after 60 days of planting.

2.4 Preparation of plant-based culture media

The succulent leaves of *Aloe vera* were washed, sliced and then blended with equal aliquots of distilled water (w/v) for 5 min in the blender. The resulting slurry homogenate was coarse filtered through cheesecloth to obtain plant juice; almost 73-82% of the plant fresh weight was recovered as juice. The pH for *Aloe vera* juices was 4-6.2. The plant juice was obtained from the tested plants were further diluted with the distilled water (v/v); 1:10, 1:20, 1:40, 1:80 and 1:100. Exclusively, such diluted juice was used as such to prepare the plant-based agar culture media (2% agar, w/v). All media were adjusted to pH 7.0 autoclaved at 1.5 atm., 121°C for 20 min, (Ibrahim *et al*., 2020).

2.5 Bio-straw (Spent mushroom compost)

To prepare grain master spawn: sorghum seeds were cleaned and then soaked in water overnight. Dead seeds were removed, then, were boiled in water for 15 min. After cooling the seeds were transferred to a round bottle (⅔ of its volume) and mixed with calcium carbonate 2 % w/w and calcium sulphate 1% w/w. Bottles were sterilized for 1 hour at 121°C. After cooling, the bottles were inoculated with mycelia discs (5 mm) diameter of 6 days old culture of white root *Pleurotus ostreatus* (was obtained from Dep. Microbiology, Soils Water and Environment Research Institute. Agricultural Research Centre (ARC), Giza, Egypt) then, the inoculated bottles were incubated at 25° C for 15-20 days. Rice straws were chopped to lengths of 2-3 cm, soaked in water (approximately three times the weight of the straw) and left for 24 hr to allow the water to completely penetrate into the straws. The soaked rice straw was transferred to autoclavable plastic bags (100 Kg/pile) which were sealed and were pasteurized (90°C for 2 hr), this process was followed by cooling to room temperature for 24 hr. After cooling, each bag was inoculated with 50 g of previously prepared spawn (culture) of *P. ostreatus*, immediately sealed. The inoculated rice straw was incubated at 30±2° C under relative humidity of 60-70% for not less than 30 days until it became well decomposed. The spent mushroom compost (SMC) and its extract chemical analysis was listed in Table (2) which was done according to standard methods as described by Brunner (1978), and Nasef *et al*. (2009).

2.6 Sampling and data collection

Three plants were randomly selected from each plot 100 days after khella fruit was sown to determine the following variables: plant height (cm), branches number/plant and herb fresh and dry weights (g)/plant. At the harvesting time on the second week of April in both seasons, the following data were recorded: number of umbels/plant, fruit yield (g)/plant, fruit yield (kg)/feddan. Also, Khellin and Visnagin percentages in the ripe dry fruits was carried out according to the method of Egyptian Pharmacopoeia (1984) with Memphis modification and total chromones percentage was calculated.

2.7 Statistical analysis

All data were tabulated and statistically analyzed according to MSTATE-C (1986) using the L.S.D. test at 5% to know the differences among all treatments according to Mead *et al*. (1993).

3. Results and Discussion

3.1 Growth parameters

Obtained data in Table (3) revealed that plant height (cm), number of branches/plant and herb fresh & dry weight (g/plant) of khella (*Ammi visnaga* L.) plants were significantly influenced by bio straw (spent mushroom compost (SMC)) treatments in the two growing seasons. It appears that fertilizing the plants with bio straw at all levels, besides the half-recommended dose of NPK except for the low one of bio straw in the first season concerning branch number led to a significant increase in plant height, number of branches/plant and herb fresh & dry weights compared to untreated plants in both seasons. From the recorded data, it is noticed that the addition of the high level of bio straw fertilizer (25 m^3/feddan) gave the highest values of these traits, whereas increased plant height, number of branches/plant and herb fresh and dry weights g/plant by 42.9 and 54.6, 108.2 and 97.1, 50.66 and 46.80, and 39.25 and 40.26% over untreated plants in the two experimental seasons, respectively. The beneficial roles of organic manures in augmenting plant growth traits were Mahmoud (2016) on khella plants, Ashwini and Jain (2017), Gahory *et al*. (2022) on *Coriandrum sativum* L. plants, Abd El-Latif (2002) on *Carum carvi*, Sharaf and Khattab (2004) on fennel, Sakr (2005) on *Cassia acutifolia* plants, Hassan *et al*. (2015) on rosemary, Hegazi *et al*. (2015) on squash plants. Concerning the effect of microbial inoculants treatments, data in Table (3) showed that plant height (cm), number of branches/plant, and herb fresh and dry weights (g)/plant of khella (*Ammi visnaga* L.) significantly increased due to the inoculation with the examined microorganisms in comparison with untreated ones in the two consecutive seasons. It was found that inoculation of khella plants with *Streptomyces rochei* (MA-6) and *Bacillus subtilis* (MA-13) in combination gave the tallest plants, highest number of branches/ plant and the heaviest herb fresh and dry weights (g)/plant as ranged 17.7 and 18.5, 33.53 and 25.3, 14.3 and 11.9, and 12.9 and 12.8% over control in the first and second seasons, respectively. The positive effect of bio-fertilization on enhancing growth parameters was observed by Hassan *et al*. (2009) on khella plant, Hussein *et al*. (2016), and Kamal and Ashish (2021) on dill (*Anethum graveolens* L.) plants, Alemu and Alemu (2015), and Somayeh and Hashem (2015) on faba bean (*Vicia faba*), Hegazi *et al*. (2015) on squash plants. According to the interaction between NPK, bio straw manure and microbial inoculant treatments, it had a significant effect on all growth parameters of khella (*Ammi visnaga* L.) plants in both seasons. Data indicated that the most effective treatments were obtained due to the high level of bio straw (25 m^3/feddan) plus a mixture of the inoculation by *Streptomyces rochei* (MA-6) and *Bacillus subtilis* (MA-13) strains as compared to other combination treatments, during the two experimental seasons, as clearly shown in Table (3).

Table (3): Effect of NPK fertilizer, bio straw levels, microbial inoculants and their interactions on the growth parameters of khella (*Ammi visnaga* L.) plants during the 2021/2022 and 2022/2023 seasons.

NPK and bio straw levels kg/feddan (A)	Microbial inoculants (B)									
	First Season					Second Season				
	Control	MI (1)	MI (2)	MI (3)	Means (A)	Control	MI (1)	MI (2)	MI (3)	Means (A)
Plant height										
Control	87.5	95.0	108.6	111.9	100.8	90.7	97.8	112.9	123.9	106.3
NPK_{HR}	117.3	128.7	128.2	135.5	127.4	126.7	136.2	143.6	153.5	140.0
15 m^3/ feddan	89.4	102.1	116.4	123.9	107.9	99.2	115.3	121.1	130.2	116.5
20 m^3/ feddan	126.3	134.5	130.2	135.4	131.6	146.7	153.6	155.5	159.3	153.8
25 m^3/ feddan	136.7	141.8	148.4	149.3	144.1	158.8	162.5	165.9	170.2	164.3
Mean(B)	111.5	120.4	126.4	131.2		124.4	133.1	139.8	147.4	
L.S.D 0.05	A = 2.1 B = 1.9 A×B = 4.3					A = 1.0 B = 0.9 A×B = 2.0				
Branch number/plant										
Control	5.4	5.5	6.4	7.2	6.1	6.4	6.6	7.2	7.9	7.0
NPK_{HR}	6.7	7.1	8.5	9.6	8.0	7.6	8.7	9.5	10.1	9.0
15 m^3/ feddan	5.2	6.1	7.0	8.4	6.7	7.1	8.1	8.8	9.6	8.4
20 m^3/ feddan	8.5	9.4	10.7	11.3	10.0	9.9	10.8	11.5	12.3	11.1
25 m^3/ feddan	11.7	12.2	13.1	13.6	12.7	12.6	13.7	14.1	14.8	13.8
Mean(B)	7.5	8.1	9.1	10.0		8.7	9.6	10.2	10.9	
L.S.D 0.05	A = 0.7 B = 0.3 A×B = 0.7					A = 1.2 B = 0.3 A×B = 0.7				
Herb fresh weight g/ plant										
Control	134.7	139.6	148.9	154.8	144.5	145.9	148.1	156.9	162.1	153.2
NPK_{HR}	159.6	161.6	169.6	170.9	165.4	163.9	165.2	175.6	177.2	170.5
15 m^3/ feddan	155.4	160.5	162.0	164.5	160.6	165.1	167.7	170.9	171.0	168.7
20 m^3/ feddan	162.4	175.2	190.9	193.6	180.5	173.4	180.4	187.4	196.9	184.5
25 m^3/ feddan	194.8	213.1	225.0	238.8	217.9	203.8	220.7	229.0	246.2	224.9
Mean(B)	161.4	170.0	179.3	184.5		170.4	176.4	184.0	190.7	
L.S.D 0.05	A = 2.7 B = 2.1 A×B = 4.7					A = 2.9 B = 2.9 A×B = 6.5				
Herb dry weight g/ plant										
Control	27.6	28.8	30.1	30.9	29.3	28.5	29.6	31.1	32.1	30.3
NPK_{HR}	31.0	33.6	34.1	35.1	33.5	31.7	33.1	34.2	34.9	33.5
15 m^3/ feddan	30.1	31.1	31.7	32.4	31.3	31.2	32.1	32.8	33.8	32.5
20 m^3/ feddan	32.8	34.7	35.3	36.5	34.8	33.7	35.2	36.8	38.4	36.0
25 m^3/ feddan	37.5	39.3	41.8	44.6	40.8	39.4	41.3	43.2	46.2	42.5
Mean(B)	31.8	33.5	34.6	35.9		32.9	34.3	35.6	37.1	
L.S.D 0.05	A = 0.9 B = 0.5 A×B = 1.1					A = 1.7 B = 0.5 A×B = 1.0				

MI (1) = *Streptomyces rochei*, MI (2) = *Bacillus subtilis* and MI (3) = MI (1) + MI (2).

3.2 Yield parameters

The data in Table (4) showed the number of umbels per plant, seed yield per plant (g), and per feddan (kg). The effect was significant on these aspects due to applying NPK and bio straw bio straw (spent mushroom compost) levels during the two study seasons. It is clear that all of them led to a significant increase in yield parameters except for NPK in the first season and the low level of bio-straw in the second season, regarding umbel number comparing to control. Moreover, the highest values of the number of umbels per plant, the seed yield per plant (g) and per feddan (kg) were obtained when khella plants were supplied with fertilizer at a high rate (25 m^3/feddan), as ranged 92.5, 57.6% concerning number of umbels per plant and 42.6 and 44.5% regarding seed yield per plant (g) and per feddan (kg), in the two seasons over the control, respectively. The effectiveness of organic manure on increasing yield parameters was revealed by Hassan *et al.* (2009) on khella plant, Kamal and Ashish (2021), and Hussein *et al.* (2016) on dill (*Anethum graveolens* L.), Ali and Hassan (2014), and Sanjeeva *et al*. (2018) on *Nigella sativa* L. plants, Abd El-Azim *et al*. (2017) on fennel, Mostafa (2018) on dragon head plant. Concerning bacterial inoculant treatments on khella (*Ammi visnaga* L.) plants, data in Table (4) showed that the influence on umbels number/plant, seed yield (g)/plant, and seed yield (kg)/feddan was significant in both seasons. From the obtained data, it is noticed that the highest umbels number/plant, seed yield (g)/plant, and seed yield (kg)/feddan were detected due to inoculating khella plants with *Streptomyces rochei* (MA-6) + *Bacillus subtilis* (MA-13) reached 44.4 and 26.1, and 14.3 and 22.0 over the check control in the two seasons, respectively.

Table (4): Effect of NPK fertilizer, bio straw levels, microbial inoculants and their interactions on the yield parameters of khella (*Ammi visnaga* L.) plants during the 2021/2022 and 2022/2023 seasons.

NPK and bio straw levels kg/feddan (A)	Microbial inoculants (B)									
	First Season					Second Season				
	Control	MI (1)	MI (2)	MI (3)	Means (A)	Control	MI (1)	MI (2)	MI (3)	Means (A)
Number of umbels/ plant										
Control	6.6	8.3	7.9	9.1	8.0	10.5	11.6	12.0	13.0	11.8
NPK_{HR}	8.4	9.7	10.2	12.3	10.2	13.9	15.9	16.3	17.7	16.0
15 m^3/ feddan	6.7	8.7	9.7	11.7	9.2	10.8	12.2	13.4	15.2	12.9
20 m^3/ feddan	11.0	13.1	11.5	14.1	12.4	15.5	17.4	17.3	18.5	17.2
25 m^3/ feddan	12.3	14.8	16.5	18.0	15.4	16.6	18.1	19.5	20.2	18.6
Mean(B)	9.0	10.9	11.2	13.0		13.4	15.1	15.7	16.9	
L.S.D 0.05	A = 1.2		B = 0.4		A×B = 0.9	A = 1.5		B = 0.5		A×B = 1.1
Seed yield (g)/plant										
Control	18.4	20.2	19.5	22.5	20.1	19.3	21.1	19.9	24.2	21.1
NPK_{HR}	21.3	22.2	23.1	23.5	22.5	21.5	23.8	23.2	24.3	23.2
15 m^3/ feddan	21.3	22.6	23.5	24.1	22.9	21.7	22.9	23.9	26.5	23.8
20 m^3/ feddan	24.1	24.8	25.6	27.5	25.5	24.2	25.2	27.3	29.5	26.6
25 m^3/ feddan	26.8	28.1	29.5	30.3	28.7	26.6	30.1	31.2	34.1	30.5
Mean(B)	22.4	23.6	24.2	25.6		22.7	24.6	25.1	27.7	
L.S.D 0.05	A = 0.8		B = 0.5		A×B = 1.1	A = 2.1		B = 1.1		A×B = 2.5
Seed yield (kg)/ feddan										
Control	612.2	672.2	648.9	750.0	670.8	642.2	703.3	662.2	805.5	703.3
NPK_{HR}	708.9	740.0	771.1	784.4	751.1	716.7	792.2	773.3	808.9	772.8
15 m^3/ feddan	711.1	754.4	783.3	802.2	762.8	724.4	762.2	797.8	884.4	792.2
20 m^3/ feddan	803.3	825.5	854.4	915.5	849.7	807.8	840.0	911.1	984.4	885.8
25 m^3/ feddan	893.3	935.5	983.3	1011.1	955.8	886.7	1002.2	1038.9	1136.7	1016.1
Mean(B)	745.8	785.5	808.2	852.7		755.5	820.0	836.7	924.0	
L.S.D 0.05	A = 25.5		B = 17.1		A×B = 38.2	A = 68.2		B = 36.9		A×B = 80.5

MI (1) = *Streptomyces rochei*, MI (2) = *Bacillus subtilis* and MI (3) = MI (1) + MI (2).

The positive effect of biofertilization on enhancing yield parameters was observed by Ali and Hassan (2014) on black cumin (*Nigella sativa* L.) plants, Hendawy *et al.* (2010) on *Thymus vulgaris* plants, Abdullah *et al*. (2012), and Hassan *et al.* (2015) on rosemary (*Rosmarinus officinalis* L.) plants. According to the interaction between NPK fertilizer, bio straw manure and microbial inoculant treatments, it had a significant effect on all yield parameters of khella (*Ammi visnaga* L.) plants in both seasons. Data indicated that the most effective treatments were obtained due to the high level of bio straw (25 m^3/feddan) plus a mixture of the inoculation by *Streptomyces rochei* (MA-6) and *Bacillus subtilis* (MA-13) strains as compared to other combination treatments, during the two experimental seasons, as clearly shown in Table (4).

3.3 Khellin, visnagin and total chromones

The recorded measurements in Table (5) showed that khellin, visnagin and total chromones percentages of khella (*Ammi visnaga* L.) were significant affected when adding NPK and bio straw (spent mushroom compost) in the two growing seasons. It is obvious that these traits were significantly increased due to the addition of NPK and bio straw except for the low one of bio straw in the two seasons concerning khellin, visnagin and total chromones percentages comparing to unfertilized plant in both seasons. This coefficient was gradually increased by increasing the bio straw fertilizer levels in the two seasons. Therefore, the highest values of khellin, visnagin and total chromones percentages, were resulted due to utilizing bio straw at the high level (25 m^3/feddan) reached 10.7 and 11.1%, 15.1 and 8.3%, and 12.0 and 10.2% over the check treatment, during the two seasons, respectively. The increments of these ingredients due to organic fertilization have been studied by Hassan *et al*. (2009) and Mahmoud (2016) on khella plants, Hemdan (2008) on anise, Abdullah *et al*. (2012) and Hassan *et al*. (2015) on rosemary, Ali and Hassan (2014) on black cumin, Mostafa (2018) on dragon head plant. Regarding the effect of microbial inoculation by *Streptomyces rochei* (MA-6) and *Bacillus subtilis* (MA-13) alone or in combination treatments, data in Table (5) showed that khellin, visnagin and total chromones percentages were significantly increased except for inoculation by *Streptomyces rochei* in the first season regarding khellin, visnagin and total chromones percentage in comparison with untreated ones in the two consecutive seasons. It was found that inoculation khella plants by *Streptomyces rochei* + *Bacillus subtilis* gave the highest increase in khellin, visnagin and total chromones percentage as ranged 7.5 and 8.2, 10.1 and 8.6, and 8.1 and 8.4% than those obtained by control in the first and second seasons, respectively .The positive effect of biofertilization on enhancing active constituents (khellin, visnagin and total chromones percentage) was observed by Hassan *et al*. (2009) on khella plant, Hendawy *et al.* (2010) on *Thymus vulgaris* plants, Ali and Hassan (2014) on black cumin (*Nigella sativa* L.) plants, Abdullah *et al*. (2012) and Hassan *et al.* (2015) on rosemary (*Rosmarinus officinalis* L.) plants. As for the interaction between the two studied factors, the effect was significant on all measurements (khellin, visnagin and total chromones percentage) of khella plants in both seasons.

Table (5): Effect of NPK fertilizer, bio straw levels, microbial inoculants and their interactions on the active constituents of khella (*Ammi visnaga* L.) plants during the 2021/2022 and 2022/2023 seasons.

NPK and bio straw levels kg/feddan (A)	Microbial inoculants (B)									
	First Season					Second Season				
	Control	MI (1)	MI (2)	MI (3)	Means (A)	Control	MI (1)	MI (2)	MI (3)	Means (A)
Khellin (%)										
Control	1.810	1.823	1.867	1.917	1.854	1.830	1.890	1.910	1.967	1.899
NPK_{HR}	1.853	1.880	1.933	1.963	1.908	1.903	1.920	1.970	2.000	1.948
15 m^3/ feddan	1.840	1.850	1.910	1.937	1.884	1.890	1.893	1.960	2.001	1.936
20 m^3/ feddan	1.917	1.967	2.033	2.117	2.008	1.947	2.060	2.097	2.150	2.063
25 m^3/ feddan	1.950	2.050	2.073	2.137	2.053	1.977	2.107	2.143	2.213	2.110
Mean(B)	1.874	1.914	1.963	2.014		1.909	1.974	2.016	2.066	
L.S.D 0.05	A = 0.033 B = 0.045 A×B = 0.101					A = 0.047 B = 0.038 A×B = 0.085				
Visnagin (%)										
Control	0.773	0.793	0.843	0.847	0.814	0.813	0.823	0.873	0.883	0.848
NPK_{HR}	0.807	0.805	0.853	0.897	0.840	0.820	0.850	0.898	0.907	0.869
15 m^3/ feddan	0.790	0.797	0.844	0.861	0.823	0.823	0.827	0.823	0.836	0.827
20 m^3/ feddan	0.847	0.863	0.863	0.942	0.879	0.850	0.887	0.890	0.927	0.888
25 m^3/ feddan	0.897	0.933	0.936	0.981	0.937	0.880	0.896	0.904	0.994	0.918
Mean(B)	0.823	0.838	0.868	0.906		0.837	0.856	0.878	0.909	
L.S.D 0.05	A = 0.026 B = 0.019 A×B = 0.042					A = 0.020 B = 0.018 A×B = 0.040				
Total chromones (%)										
Control	2.58	2.62	2.71	2.76	2.67	2.64	2.71	2.78	2.85	2.75
NPK_{HR}	2.66	2.68	2.79	2.86	2.75	2.72	2.77	2.87	2.91	2.82
15 m^3/ feddan	2.63	2.65	2.75	2.80	2.71	2.71	2.72	2.78	2.84	2.76
20 m^3/ feddan	2.76	2.83	2.90	3.06	2.89	2.80	2.95	2.99	3.08	2.95
25 m^3/ feddan	2.85	2.98	3.01	3.12	2.99	2.86	3.00	3.05	3.21	3.03
Mean(B)	2.70	2.75	2.83	2.92		2.75	2.83	2.89	2.98	
L.S.D 0.05	A = 0.08 B = 0.06 A×B = 0.13					A = 0.06 B = 0.04 A×B = 0.09				

MI (1) = *Streptomyces rochei*, MI (2) = *Bacillus subtilis* and MI (3) = MI (1) + MI (2).

The data indicated that the most effective treatments were obtained by adding a high rate of bio straw (25 m^3/feddan) with double inoculation by *Streptomyces rochei* (MA-6) and *Bacillus subtilis* (MA-13) as compared to other treatments for both seasons, as shown in Table (5). From the obtained results, it could be recommended to supply the soil of khella (*Ammi visnaga* L.) plants with bio straw (25 m^3/feddan) plus a mixture of the inoculation by *Streptomyces rochei* (MA-6) and *Bacillus subtilis* (MA-13) to enhance the growth, yield and active constituents (khellin, visnagin and total chromones percentage) under the conditions of this work.

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