Effect ofinorganic fertilizers and biofertilizers on growth, herb yield and quality of Japanese mint (*Mentha arvensis* L.) var. Kosi.

Abstract :

Aim: To study about the effect ofinorganic fertilizers and biofertilizerson growth, herb yield and menthol content of Japanese mint.

Study Design: The experiment was carried out with 13 treatments in Randomized Block Design (RBD) with three replications.

Place and Duration of Study: Research trial was carried out at P.G block, College of Horticulture, Rajendranagar, SKLTSHU, Hyderabad during Feb-May 2022 and Aug- Nov 2022

Results: Among the different treatments, the results reported that the T_2 (100% RDF + *Azospirillum*)recorded maximun plant height (55.53 cm and 54.20 cm), number of primary branches (26.40 and 25.73), number of secondary branches (8.27 and 8.13), number of leaves (947.53 and 937.20), leaf area (18.37 cm^2 and 18.03 cm^2) , fresh herb yield per hectare(225.04 q and 221.34 q)and essential oil yield per hectare (205.69 kg and 184.15 kg) compared to other treatments which was statistically on par with T_1 (100% RDF + Azotobacter), T_6 (75% RDF + Azospirillum) and T_5 (75% RDF + Azotobacter).There by it can be concluded that we can decrease the inorganic fertilizer dose upto 25 % by adding azospirillum or azototobacter to get the optimum herb yield and essential oil yield in Japanese mint.

Key words: Japanese mint; *azotobacter*; *azospirillum*; kosi.

1. INTRODUCTION:

"Mint is the common name of *Mentha arvensis* L. having approximately 25 species of the genus *Mentha* belonging to family Lamiaceae. The mint species *Mentha arvensis,* termed as Japanese mint have 80-85% menthol contents" (Ozguven and Kirici, 1999). "Kosi is a new released, early variety of menthol mint (90-100 days) and produces higher oil content (0.3-1.2%) containing 81- 83% menthol" (Verma *et al.* 2018)."The crop is commercially cultivated as spring season crop (January–February to April–May) in Tarai and the central part of Uttar Pradesh and Uttarakhand, Punjab, Bihar and Haryana" (Singh *et al.* 1998; Upadhyay *et al.* 2012).Mint, either as herb or its essential oil form is used for flavoring, perfume production and medicinal purposes (Dorman *et al.,* 2003)."Mint is valued for its multipurpose uses in the field of pharmaceuticals, cosmetics as well as for flavoring foods, beverages and tobacco" (Ohloff, 1994)."Essential oil and their valuable chemical constituents obtained from menthol mint have great export potential. Higher essential oil content and better quality of oil will give higher production and more output to the producers.The area under menthol mint cultivation in India is estimated to be 0.15 million hectares with annual production of 20,000 metric tons of essential oil.The use of biofertilizers containing beneficial soil microorganisms as alternative for chemical fertilizers is an effective way to improve plant growth, environment health and soil productivity.Bio-fertilizers help in better utilization of added inorganic fertilizers thus reduce its level of application as well as reduce the deleterious effect of harsh chemical residues that the inorganic fertilizers leave in the soil" (Umar, 2007)."Nitrogen (N) is one of the principal plant nutrients for improved plant growth and yield" (Ayub et al., 2010). "However, the global nitrogen cycle pollutes groundwater and increases the risk of chemical spills and its low availability due to the high losses by emission or leaching is a limiting factor in agricultural ecosystems, hence bacteria with ability to make atmospheric N available for plants play a critical role reducing the need for chemical fertilizers and decreasing their adverse environmental effects. available Biological nitrogen fixation fixes about 60% of the earth's nitrogen and represents an economically beneficial and environmentally sound alternative to chemical fertilizers" (Ladha and Kundu, 1997). . "Bio fertilizers can influence plant growth directly through the production of phytohormones such as gibberellins, cytokinins and IAA that act as growth regulators and indirectly through nitrogen fixation and production of bio-control agents against soil-borne Phytopathogens and consequently increase formation of metabolites which encourage the plant vegetative growth and enhance the meristematic activity of tissues to produce more growth" (Glick, 2003 and Ahmed and Kibret, 2014) "Utilization of biofertilizers enhance productivity by biological nitrogen fixation or solubilization of insoluble phosphate or producing harmones, vitamins and other growth factors required for plant growth" (Bhattacharyya *et al.* 2000)

2. MATERIAL AND METHODS:

2.1 THE EXPERIMENTAL SITE

The present investigation was conducted at College of Horticulture, Rajendranagar during Feb-May and Aug-Nov 2022. The experimental site is situated at a latitude of 17°.32' North, longitude of 78°.40' East and altitude of 542.3 m above mean sea level.

2.2 Chart1 : THE EXPERIMENT DESIGN

T_1	100% RDF + *Azotobacter*
T_2	100% RDF + *Azospirillum*
T_3	100% RDF + *VAM*
T_4	100% RDF + *PSB*
T_5	75% RDF + *Azotobacter*
T_6	75% RDF + *Azospirillum*
T_7	75% RDF + *VAM*
T_8	75% RDF + *PSB*
T_9	50% RDF + *Azotobacter*
T_{10}	50% RDF + *Azospirillum*
T_{11}	50% RDF + *VAM*
T_{12}	50% RDF + *PSB*
T_{13}	Control (RDF- 160: 50: 40 NPK kg/ha)

2.3 THE PARAMETERS DETERMINED

2.3.1 Growth parameters

2.3.1.1. Plant height

Plant height was measured from the base to the tip of the plant from five randomly tagged plants in each treatment at 30, 60, 90 days after planting (DAP) and at final harvest and their mean values were worked out and presented.

2.3.1.2 Number of primary branches

Number of primary branches of the plant was counted from five randomly tagged plants in each treatment at 60, 90 days after planting (DAP) and at final harvest and their mean values were worked out and presented.

2.3.1.3 Number of secondary branches

Number of secondary branches of the plant was counted from five randomly tagged plants in each treatment at 90 and 120 days after planting and at final harvest and their mean values were worked out and presented.

2.3.1.4. Number of leaves

Number of leaves of the plant was counted from five randomly tagged plants in each treatment at 30, 60, 90 days after planting (DAP) and at final harvest and their mean values were worked out and presented.

2.3.1.5. Leaf Area (LA)

Leaves of five plants selected for fresh herb weight were separated and the leaf area was measured on LI-3100 Leaf area meter at 30, 60, 90 days after transplanting and at harvest and expressed as cm^2.

2.3.1.6. Crop Growth Rate (CGR)

The dry matter accumulation per unit land area is called as crop growth rate. After calculating the dry weight per plant, crop growth rate per plant was calculated at 60, 90 DAP and at final harvest. The CGR was calculated by the formula.

$$CGR = \frac{W_2 - W_1}{t_2 - t_1} \times \frac{1}{P}$$

Where, W_1 and W_2 are dry weight of the whole plant at time t_1 and t_2 respectively per unit land area (P).

2.3.2 Yield parameters

2.3.2.1. Fresh herb yield per hectare

Fresh herbage weight from each plot was calculated by separating the herb from the root of the entire plants was converted to per hectare and it was expressed in quintals (q).

2.3.2.2. Essential oil content

Essential oil from fresh herbage was extracted by using Clevenger apparatus. One kilogram of fresh herbage was chopped and taken into the mantle. One litre of water was added to the mantle and boiled at 90°C. The percentage of oil extracted from fresh herbage was calculated by using the following formula.

$$\text{Essential oil (\%)} = \frac{\text{Weight of the oil}}{\text{Weight of the herbage}} \times 100$$

2.3.2.3. Oil yield

Oil yield was calculated by multiplying the fresh herb yield at harvest with respective essential oil content obtained by distillation process (on fresh weight basis) and expressed as litre/ha.

$$\text{Oil yield (Kg/ha)} = \frac{\text{Oil content (\%)} \times \text{Fresh herbage yield}}{100}$$

2.3.2.3.1 Extraction of oil from Japanese mint by Clevenger's Apparatus

Oil from fresh herbage was extracted by using clevenger's apparatus. Apparatus consist of one round bottom flask of 1000 ml which is connected with another two way round flask which holds raw material. The top flask is connected with condenser through the connecter. The separating funnel is used for the separation of essential oil and water (Shahin, 2017.)

Oil extraction Procedure

Fresh shoots of Japanese mint along with leaves are cut into pieces within half a day after collection. The cut herbage of 250-500 g boiled with 1000 ml of distilled water in a Clevenger apparatus until oil distillation ceased after 5-6 hours. The volume of essential oil (ml) was determined from a calibrated trap. The essential oil in the distillate was dried over anhydrous Na_2SO_4 and kept in the freezer.

2.3.2.4 Menthol content

The mint oil was subjected to Gas chromatography using Flame Ionization detector fitted with electronic integrator using a 25 mm BP-20 fused silica column.

The Gas chromatography capillary column (30 m × 0.25 mm, 0.25 µm) programmed with temperature from 60 to 230^{o}C at 3ºC/min and then at 70 to 230ºC at 4ºC/min. Hydrogen gas was the carrier gas with injection volume 0.02 µl. Identification was based on retention time of standard compounds. The relative amount of individual components were calculated based on percent pack area relative to total peak area from the GC/FID analyses without using correction factor. Menthol content were expressed in percentage (%).

2.4 STATISTICAL ANALYSIS

The experiment was laid out in randomized block design (RBD) with 13 treatments and 3 replications. The experimental data collected on various growth, flowering and yield components of plant were subjected to Fisher's method of "Analysis of variance" (ANOVA) as outlined by [7] were analysed.

3. RESULTS AND DISCUSSION

The results of the present investigation regarding the response of inorganic fertilizers and biofertilizers on growth, yield and economics of Japanese mint have been discussed and interpreted in light of previous research work in India. The results of the experiment are summarized below and also presented in tables.

3.1 Growth parameters

3.1.1 Plant height

The plant height differed significantly among the treatments and increased with advancement of crop stage.

The data revealed that during season 1, at 30, 60, 90 and 120 DAP, among the treatments, highest plant height (26.17 cm, 40.60 cm, 49.83 cm and 55.53 cm respectively) was observed with T_2 (100% RDF + *Azospirillum*) which was statistically on par with T_1 (100% RDF + *Azotobacter*), T_6 (75% RDF + *Azospirillum*) and T_5 (75% RDF + *Azotobacter*). The lowest plant height (15.30 cm, 30.87 cm, 35.73 cm and 41.40 cm) at 30, 60, 90 and 120 DAP was recorded in T_{11} (50% RDF + *VAM)*.

The data presented in table 1 revealed that during season 2, at 30, 60, 90 and 120 DAP, among the treatments, the highest plant height (24.83 cm, 39.27 cm, 48.50 cm and 54.00 cm respectively) was observed with T_2 (100% RDF + *Azospirillum*) which was statistically on par with T_1 (100% RDF + *Azotobacter*), T_6 (75% RDF + *Azospirillum*) and T_5 (75% RDF + *Azotobacter*). The lowest plant height (14.57 cm, 29.53 cm, 34.40 cm and 40.07 cm) at 30, 60, 90 and 120 DAP was recorded in T_{11} (50% RDF + *VAM)*.

In both the seasons T_2 (100% RDF + *Azospirillum*) recorded maximum plant height. The highest plant height could be due to certain growth promoting substances secreted by biofertilizers i.e., *Azospirillum* and *Azotobacter* which in turn, might have led to better root development, better transportation of water, uptake and deposition of nutrients. This may be due to application of integrated nutrient management, increased the photosynthetic activity, chlorophyll formation, nitrogen metabolism and auxin contents in the plants which ultimately improving the plant height. These findings were in agreement with the findings of Khandeel *et al*. (2002)

in *Ocimum basillicum*, Apurva *et al*. (2017) in *Rauwolfia serpentina* and Aswani *et al*. (2020) in Japanese mintSwetha et al. (2021) in kalmeghwho reported that highest plant height was observed with the treatment of biofertilizer in comparison to the other treatments plants.

Table1. Effect of inorganic fertilizers and biofertilizers on plant height (cm) of Japanese mint (*Menthaarvensis* L.) var. Kosi during Feb-May and Aug-Nov 2022

Treatments	**30 DAP**		**60 DAP**		**90 DAP**		**120 DAP**	
	Feb-May 2022	**Aug-Nov 2022**	**Feb-May 2022**	**Aug-Nov 2022**	**Feb-May 2022**	**Aug-Nov 2022**	**Feb-May 2022**	**Aug-Nov 2022**
T_1: 100% RDF+Azotobacter	25.87	24.47	39.63	38.40	48.67	47.33	54.17	53.03
T_2 : 100% RDF + Azospirillum	26.17	24.83	40.60	39.27	49.83	48.50	55.53	54.20
T_3 : 100% RDF + VAM	23.63	22.23	36.43	35.03	43.50	42.17	48.57	47.23
T_4 : 100% RDF + PSB	22.83	21.50	36.20	34.87	42.53	41.20	47.20	45.87
T_5 : 75% RDF + Azotobacter	25.00	23.60	37.40	37.20	46.96	45.63	52.20	51.60
T_6 : 75% RDF + Azospirillum	25.23	23.90	37.53	38.30	47.17	45.83	53.00	52.33
T_7 : 75% RDF + VAM	21.07	20.33	33.53	32.20	38.37	37.03	44.90	43.57
T_8 : 75% RDF + PSB	20.97	20.30	33.10	31.77	38.50	37.17	44.73	43.40
T_9 : 50% RDF + Azotobacter	15.63	14.90	31.80	30.47	36.53	35.20	41.87	40.53
T_{10} : 50% RDF + Azospirillum	15.23	14.57	30.70	29.37	35.27	33.93	42.30	40.97
T_{11} : 50% RDF + VAM	15.30	14.57	30.87	29.53	35.73	34.40	41.40	40.07
T_{12} : 50% RDF + PSB	15.20	14.53	30.96	29.63	35.97	34.63	41.07	39.73
T_{13} : Control (RDF-160:50:40 NPK kg/ha)	21.67	20.93	35.73	34.40	42.37	41.03	46.33	45.00
SEm±	0.57	0.49	1.21	0.87	1.50	1.04	1.56	0.99
CD @ 5%	1.67	1.42	3.53	2.53	4.37	3.05	4.55	2.88

3.1.2. Number of primary branches per plant

The perusal of data pertaining to number of primary branches per plant during season 1 indicated that significantly maximum number of branches (11.07, 26.40 and 27.07) at 60, 90 and 120 DAP respectively was observed with the application of T_2 (100% RDF + *Azospirillum*) and was at par with T_1 (100% RDF + *Azotobacter*), T_6 (75% RDF + *Azospirillum*) and T_5 (75% RDF + *Azotobacter*). The minimum number of branches per plant (6.14, 18.23 and 20.83) was recorded with T_{11}(50% RDF + *VAM)*.

The perusal of data pertaining to number of primary branches per plant during season 2 indicated that significantly maximum number of primary branches (10.40, 25.73 and 27.07) at 30, 60, 90 and 120 DAP respectively was observed with the application of T_2 (100% RDF + *Azospirillum*) and was at par with T_1 (100% RDF + *Azotobacter*), T6 (75% RDF + *Azospirillum*) and T_5 (75% RDF + Azotobacter). The minimum number of primary branches per plant (5.48, 16.90 and 20.16) respectively was recorded with T_{11} (50% RDF + *VAM*).

In the present study maximum number of primary branches per plant was recorded in T_2 (100% RDF + *Azospirillum*) which may be due to combined application of inorganic fertilizers and biofertilizer, which influenced the availability of nitrogen. Azospirillium 20g /kg had significant influence on growth and quality attributes of the plant, this might be due to the increased uptake of available major nutrients to the plant which results in the translocation of nutrients to the plant parts. It can fix large amount of nitrogen which is freely available at atmosphere and also involved in protein synthesis, increased activity of Gibberellic acid, indole acetic acid and dehydrogenase activity, which might have improved the number of primary branches per plant.These results were in conformity with Singh *et al* (2012) in stevia, Roshan *et al.* (2014) in coriander.

Table 2. **Effect of inorganic fertilizers and biofertilizers on number of primary branches of Japanese mint (*Menthaarvensis* L.) var. Kosi during Feb-May and Aug-Nov 2022.**

Treatments	**60 DAP**		**90 DAP**		**120 DAP**	
	Feb-May 2022	**Aug-Nov 2022**	**Feb-May 2022**	**Aug-Nov 2022**	**Feb-May 2022**	**Aug-Nov 2022**
T_1: 100% RDF+Azotobacter	10.80	10.13	26.50	25.17	25.73	25.07
T_2 : 100% RDF + Azospirillum	11.07	10.40	27.07	25.73	26.40	25.73
T_3 : 100% RDF + VAM	9.07	8.40	21.03	20.13	23.73	23.07
T_4 : 100% RDF + PSB	8.87	8.20	21.47	19.70	23.67	23.00
T_5 : 75% RDF + Azotobacter	9.80	9.12	24.40	23.17	24.23	23.93
T_6 : 75% RDF + Azospirillum	9.90	9.23	24.60	23.54	25.13	24.47
T_7 : 75% RDF + VAM	7.40	6.73	20.93	19.60	22.10	21.43
T_8 : 75% RDF + PSB	7.20	5.87	20.83	19.50	21.84	21.17
T_9 : 50% RDF + Azotobacter	7.03	6.37	19.23	17.90	21.14	20.47
T_{10} : 50% RDF + Azospirillum	6.53	5.87	18.73	17.40	21.02	20.35
T_{11} : 50% RDF + VAM	6.14	5.48	18.23	16.90	20.83	20.16
T_{12} : 50% RDF + PSB	5.81	5.15	17.53	16.20	20.43	19.76
T_{13} : Control (RDF-160:50:40 NPK kg/ha)	9.33	8.67	20.63	19.30	22.59	21.92
SEm±	0.46	0.46	0.99	0.94	0.68	0.67
CD @ 5%	1.35	1.33	2.90	2.75	1.98	1.97

3.1.3 Number of secondary branches per plant

The perusal of data pertaining to number of secondary branches per plant during season 1 indicated that significantly maximum number of secondary branches (4.70 and 8.27) at 90 and 120 DAP respectively was observed with the application of T$_2$ (100% RDF + *Azospirillum*)which was statistically on par with T$_1$ (100% RDF + *Azotobacter*), T$_6$ (75% RDF + *Azospirillum*) and T$_5$ (75% RDF + *Azotobacter*. The minimum number of branches per plant (1.00 and 3.67) respectively were recorded with T$_{11}$(50% RDF + *VAM*).

The perusal of data pertaining to number of secondary branches per plant during season 2 indicated that significantly maximum number of secondary branches (4.57 and 8.13) at 90 and 120 DAP respectively was observed with the application of T$_2$ (100% RDF + *Azospirillum*) which was statistically on par with T$_1$ (100% RDF + *Azotobacter*), T$_6$ (75% RDF + *Azospirillum*) and T$_5$ (75% RDF + *Azotobacter*). The minimum number of primary branches per plant (1.00 and 3.53) at 90 and 120 DAP respectively was recorded with T$_{11}$ (50% RDF + *VAM*).

In the present study maximum number of secondary branches per plant was recorded in T$_2$ (100% RDF + *Azospirillum*) which may be due to combined application of recommended dose of fertilizer and Azospirillum which has capability of fixing atmospheric nitrogen inturn increased the availability of nitrogen. As azospirillum helps in production of more amount of nitrogen availability to plants. Similar trend was observed by Khandeel *et al*. (2002) in *Ocimum basillicum*, Singh *et al* (2012) in stevia.

Table 3.Effect of inorganic fertilizers and biofertilizers on number of secondary branches of Japanese mint (*Menthaarvensis* L.) var. Kosi during Feb-May and Aug-Nov 2022.

Treatments	90 DAP		120 DAP	
	Feb-May 2022	Aug-Nov 2022	Feb-May 2022	Aug-Nov 2022
T$_1$: 100% RDF+Azotobacter	4.43	4.30	7.87	7.73
T$_2$: 100% RDF + Azospirillum	4.70	4.57	8.27	8.13
T$_3$: 100% RDF + VAM	3.50	3.39	6.37	6.23
T$_4$: 100% RDF + PSB	3.53	3.43	6.43	6.30
T$_5$: 75% RDF + Azotobacter	4.13	4.01	7.30	7.17
T$_6$: 75% RDF + Azospirillum	4.23	4.12	7.50	7.40
T$_7$: 75% RDF + VAM	3.00	2.87	5.87	5.73
T$_8$: 75% RDF + PSB	2.90	2.77	5.67	5.53
T$_9$: 50% RDF + Azotobacter	1.80	1.67	3.87	3.73
T$_{10}$: 50% RDF + Azospirillum	1.10	1.07	3.77	3.63
T$_{11}$: 50% RDF + VAM	1.00	1.00	3.67	3.53
T$_{12}$: 50% RDF + PSB	1.00	1.00	3.67	3.53
T$_{13}$: Control (RDF-160:50:40 NPK kg/ha)	3.57	3.43	6.27	6.13
SEm±	0.26	0.20	0.36	0.38
CD @ 5%	0.77	0.57	1.04	1.10

3.1.4. Number of leaves per plant

The data revealed that during season 1 maximum number of leaves per plant (30.60, 257.53, 760.87 and 947.53) at 30, 60, 90 and 120 DAP respectively was observed with T_2 (100% RDF + *Azospirillum*), and was statistically on par with T_1 (100% RDF + *Azotobacter*), T_6 (75% RDF + *Azospirillum*) and T_5 (75% RDF + *Azotobacter*). The minimum number of leaves per plant (25.67, 109.43, 459.12 and 602.10)respectively was recorded with T_{11} (50% RDF + *VAM*).

The data presented in Table 4. revealed that during season 2 maximum number of leaves per plant (29.93, 250.87, 755.87 and 937.20) at 30, 60, 90 and 120 DAP respectively was observed with T_2 (100% RDF + *Azospirillum*), and was statistically on par with T_1 (100% RDF + *Azotobacter*), T_6 (75% RDF + *Azospirillum*) and T_5 (75% RDF + *Azotobacter*). The minimum number of leaves per plant (25.00, 106.09, 454.12 and 590.77) respectively was recorded with T_{11} (50% RDF + *VAM*).

The higher number of leaves per plant during season 1 and season 2 was obtained under the treatment T_2 i.e., combined application of inorganic and biofertilizers (100% RDF + *Azospirillum*) The result revealed that the combined application of inorganic fertilizers and *Azospirillum* recorded significantly maximum values at all the growth stages could be due to timely supply of all nutrients, resulted in luxurious vegetative growth of plant. Increased number of leaves are also related to Azospirillum applied which might have fixed atmospheric nitrogen and produced bioactive substances having similar effects as that of growth regulators which in turn has resulted in increasing the number of leaves. These findings were in agreement with the findings of Roshan *et al.* (2014) in coriander, Aswani*et al*. (2020) in Japanese mint, Anjali *et al.* (2021)in lemon grass and Sai krishna *et al.* (2022) in palak.

Table 4. Effect of inorganic fertilizers and biofertilizers on number of leaves of Japanese mint (*Menthaarvensis* L.) var. Kosi during Feb-May and Aug-Nov 2022.

Treatments	30 DAP		60 DAP		90 DAP		120 DAP	
	Feb-May 2022	**Aug-Nov 2022**	**Feb-May 2022**	**Aug-Nov 2022**	**Feb-May 2022**	**Aug-Nov 2022**	**Feb-May 2022**	**Aug-Nov 2022**
T_1: 100% RDF+Azotobacter	28.40	27.73	240.20	236.87	748.20	743.20	921.87	911.53
T_2 : 100% RDF + Azospirillum	30.60	29.93	257.53	250.87	760.87	755.87	947.53	937.20
T_3 : 100% RDF + VAM	26.63	25.30	194.97	191.63	645.30	640.30	749.07	737.73

T_4 : 100% RDF + PSB	26.93	25.60	218.63	215.30	638.40	633.40	762.63	751.63
T_5 : 75% RDF + Azotobacter	27.67	27.00	233.83	230.49	735.20	730.20	894.63	886.97
T_6 : 75% RDF + Azospirillum	28.19	27.53	235.20	231.87	738.63	733.63	902.20	894.87
T_7 : 75% RDF + VAM	25.73	25.07	179.07	175.73	565.87	560.87	652.63	641.96
T_8 : 75% RDF + PSB	25.20	24.53	172.53	169.20	554.30	549.30	648.63	637.63
T_9 : 50% RDF + Azotobacter	25.30	24.63	110.97	107.63	472.83	467.83	606.17	595.17
T_{10} : 50% RDF + Azospirillum	25.67	25.00	109.50	106.16	468.77	463.77	605.09	594.42
T_{11} : 50% RDF + VAM	25.67	25.00	109.43	106.09	459.12	454.12	602.10	590.77
T_{12} : 50% RDF + PSB	25.07	24.40	95.20	91.87	457.17	452.17	598.50	586.83
T_{13} : Control (RDF-160:50:40 NPK kg/ha)	26.10	25.43	165.93	162.60	639.27	634.27	679.60	675.60
SEm±	1.09	1.30	9.86	7.56	12.42	11.07	19.16	19.26
CD @ 5%	3.17	3.78	28.79	22.07	36.26	32.32	55.91	56.21

3.1.5 Leaf area (cm^2)

Data with regard to leaf area (cm^2) are furnished in Table 5. Leaf area was significantly influenced due to inorganic manures and biofertilizers in combined application at 30, 60, 90 and 120 DAP.

The perusal of data pertaining to leaf area during season 1 indicated that significantly maximum leaf area (3.97 cm^2, 8.12 cm^2, 13.05 cm^2 and 18.37 cm^2) at 30, 60, 90 and 120 DAP respectively was observed with application of T_2 (100% RDF + *Azospirillum*), and was statistically on par with T_1 (100% RDF + *Azotobacter*), T_6 (75% RDF + *Azospirillum*) and T_5 (75% RDF + *Azotobacter*). The minimum leaf area (3.10 cm^2, 4.10 cm^2, 8.00 cm^2 and 13.80 cm^2) respectively was recorded with T_{12} (50% RDF + *PSB)*.

The data presented in Table 5. revealed that during season 2 significantly maximum leaf area (3.90 cm^2, 7.98 cm^2, 12.91 cm^{2}and 18.03 cm^2) at 30, 60, 90 and 120 DAP respectively was observed with application of T_2 (100% RDF + *Azospirillum*) and was statistically on par with T_1 (100% RDF + *Azotobacter*), T_6 (75% RDF + *Azospirillum*) and T_5 (75% RDF + *Azotobacter)*. The minimum leaf area (3.03 cm^2, 3.97 cm^2, 7.86 cm^2 and 13.47 cm^2) respectively was recorded with T_{12} (50% RDF + *PSB*).

The leaf area (cm^2) recorded was more in T_2 at 30, 60, 90, and 120 DAP during season 1 and season 2 might be due to higher availability of nutrients which have accelerated the synthesis of chlorophyll and amino acids which are associated with photosynthetic process of plants resulted in higher leaf area. These findings are in agreement with the findings of Khandeel *et al.* (2002) in *Ocimum basillicum* and Roshan *et al.* (2014) in coriander.

Table 5. Effect of inorganic fertilizers and biofertilizers on leaf area (cm^2) of Japanese mint (*Menthaarvensis* L.) var. Kosi during Feb-May and Aug-Nov 2022.

Treatments	**30 DAP**		**60 DAP**		**90 DAP**		**120 DAP**	
	Feb-May 2022	**Aug-Nov 2022**	**Feb-May 2022**	**Aug-Nov 2022**	**Feb-May 2022**	**Aug-Nov 2022**	**Feb-May 2022**	**Aug-Nov 2022**
T_1: 100% RDF+Azotobacter	3.94	3.88	8.07	7.94	12.97	12.83	17.87	17.53
T_2 : 100% RDF + Azospirillum	3.97	3.90	8.12	7.98	13.05	12.91	18.37	18.03
T_3 : 100% RDF + VAM	3.72	3.66	6.62	6.56	10.70	10.57	16.49	16.16
T_4 : 100% RDF + PSB	3.80	3.73	6.86	6.69	10.72	10.59	16.82	16.49
T_5 : 75% RDF + Azotobacter	3.91	3.85	7.23	7.30	12.20	12.06	17.12	16.79
T_6 : 75% RDF + Azospirillum	3.92	3.85	7.96	7.83	12.25	12.11	17.50	17.17
T_7 : 75% RDF + VAM	3.68	3.62	6.16	6.03	9.39	9.26	15.81	15.48
T_8 : 75% RDF + PSB	3.64	3.57	6.16	6.03	9.49	9.35	15.68	15.35
T_9 : 50% RDF + Azotobacter	3.41	3.35	5.63	5.49	8.27	8.14	14.56	14.23
T_{10} : 50% RDF + Azospirillum	3.37	3.30	5.41	5.28	8.30	8.17	14.80	14.47
T_{11} : 50% RDF + VAM	3.23	3.17	4.40	4.27	7.70	7.57	14.42	14.09
T_{12} : 50% RDF + PSB	3.10	3.03	4.10	3.97	8.00	7.86	13.80	13.47
T_{13} : Control (RDF-160:50:40 NPK kg/ha)	3.44	3.37	6.88	6.44	9.88	9.75	15.61	15.28
SEm±	0.205	0.05	0.32	0.26	0.39	0.42	0.47	0.44
CD @ 5%	0.598	0.14	0.94	0.75	1.13	1.21	1.36	1.28

3.1.6 Crop growth rate (g m^{-2} d^{-1})

The data enunciated on crop growth rate (g m^{-2} d^{-1}) at 30 - 60, 60 - 90 and 90 - 120 DAP as affected by the application of inorganic fertilizers and biofertilizers during season 1 and season 2 are presented in the Table 6.

With respect to different treatments during season 1 there were significant differences on crop growth rate. significantly maximum crop growth rate (1.60, 4.11 and 2.23 g m^{-1} day) at 30-60, 60-90 and 90-120 DAP respectively was observed with application of T_2 (100% RDF + *Azospirillum*), and it was statistically on par with T_1

(100% RDF + *Azotobacter*),T_6 (75% RDF + *Azospirillum*) and T_5 (75% RDF + Azotobacter*)*. The minimum crop growth rate (1.46, 3.34 and 1.12 g m^{-1} day) was recorded with T_{11} (50% RDF + *VAM*).

The crop growth rate (CGR) was determined from 30 to 120 DAP and the results are furnished in Table 6.During season 2 significantly maximum crop growth rate (1.55, 4.08 and 2.02) at 30-60, 60-90 and 90-120 DAP respectively was observed with application of T_2 (100% RDF + *Azospirillum*) and was statistically on par with T_1 (100% RDF + *Azotobacter*), T_6 (75% RDF + *Azospirillum*) and T_5 (75% RDF + *Azotobacter).* The minimum crop growth rate (1.44, 3.19 and 0.87 g m^{-1} day) was recorded with T_{11} (50% RDF + *VAM*).

Dry matter produced for unit area during a particular time interval (CGR) is a product of NAR and LAI. It presents sum of CGR's of individual component. Major plant determinant of photosynthetic potential is developed and maintenance of photosynthetically active leaf area. The increasing crop growth rate due to combined application of inorganic fertilizers and biofertilizers may be due to the ready availability of macronutrients and nitrogen fixation by azospirillum and azotobacter there by increase in number of leaves, fresh weight and dry weight of leaves which increased the leaf area and leaf area index in treatment T_2 (100% RDF + *Azospirillum*) during season 1 and season 2.These findings were in agreement with the findings of Khandeel *et al.* (2002) in *Ocimum basillicum* and Singh *et al.* (2012) in stevia.

Table 6. Effect of inorganic fertilizers and biofertilizers on crop growth rate (g m^{-1} day^{-1}) of Japanese mint (*Menthaarvensis* L.) var. Kosi during Feb-May and Aug-Nov 2022.

Treatments	30- 60 DAP		60- 90 DAP		120 DAP	
	Feb-May 2022	Aug-Nov 2022	Feb-May 2022	Aug-Nov 2022	Feb-May 2022	Aug-Nov 2022
T_1: 100% RDF+Azotobacter	1.59	1.54	3.94	3.92	2.13	1.93
T_2 : 100% RDF + Azospirillum	1.60	1.55	4.11	4.08	2.23	2.02
T_3 : 100% RDF + VAM	1.53	1.51	3.73	3.68	1.52	1.27
T_4 : 100% RDF + PSB	1.51	1.49	3.74	3.69	1.63	1.39
T_5 : 75% RDF + Azotobacter	1.56	1.53	3.79	3.74	1.98	1.78
T_6 : 75% RDF + Azospirillum	1.57	1.53	3.81	3.76	2.06	1.81
T_7 : 75% RDF + VAM	1.46	1.44	3.39	3.34	1.39	1.14
T_8 : 75% RDF + PSB	1.51	1.48	3.34	3.29	1.36	1.12
T_9 : 50% RDF + Azotobacter	1.51	1.48	3.29	3.24	1.21	0.97
T_{10} : 50% RDF + Azospirillum	1.51	1.48	3.32	3.27	1.29	1.04
T_{11} : 50% RDF + VAM	1.46	1.44	3.34	3.19	1.12	0.87
T_{12} : 50% RDF + PSB	1.51	1.48	3.34	3.29	1.19	0.94
T_{13} : Control (RDF-160:50:40 NPK kg/ha)	1.46	1.44	3.59	3.54	1.44	1.19
SEm±	0.02	0.01	0.12	0.13	0.12	0.09
CD @ 5%	0.05	0.03	0.34	0.37	0.36	0.26

3.1.7 Fresh herb yield per hectare (q)

Data on the fresh herb yield per hectare at harvest as influenced by different treatments are presented in Table 7. Significantly maximum fresh herb yield per hectare (225.04 q) was recorded in T_2 (100% RDF + *Azospirillum*), and was statistically on par with T_1 (100% RDF + *Azotobacter*), T_6 (75% RDF + *Azospirillum*) and T_5 (75% RDF + *Azotobacter)*. The minimum fresh herb yield per hectare (144.08 q) at 30, 60, 90 and 120 DAP respectively was recorded with T_{11} (50% RDF + *VAM*) during season 1.

With respect to different treatments, fresh herb yield per hectare was significantly affected at harvest. The treatment T_2 (100% RDF + *Azospirillum*) registered significantly maximum fresh herb yield per hectare (221.34 q) and was on par with T_1 (100% RDF + *Azotobacter*), T_6 (75% RDF + *Azospirillum*) and T_5 (75% RDF + *Azotobacter*. The minimum fresh herb yield per hectare (139.78 q) at 30, 60, 90 and 120 DAP respectively was recorded with T_{11} (50% RDF + *VAM*) during season 2.

The increased fresh yield per hectare with application of 100% RDF + *Azospirillum* (T_2) in season 1 and season 2 could be attributed to increased plant height, number of branches, plant spread, number of leaves, leaf area and dry matter accumulation with this treatment. It is further evident from the data that use of*Azospirillum* in combination with RDF influenced the herbage yield of Japanese mint. The effects of Azospirillum to induce plant growth were mainly associated with the capacity to fixN_2 and it is known that this bacterium has different additional mechanisms that contribute to greater growth and crop yield. Among them, the phosphate solubilization, hormone and/or siderophore production (Puente et al., 2008), phytopathogens control (Bashan and de-Bashan, 2010), and protection against abiotic stress like drought, salinity, or toxic compounds have been characterized. This might be due to the availability of the nutrients in readily available form. These findings were in agreement with the finding ofSindhu et al. (2016) in*Indigofera tinctoria*Aswani *et al*. (2020) in Japanese mint.

3.1.8 Essential oil content (%)

The data pertaining to essential oil content (%) influenced by the application of inorganic fertilizers and biofertilizers during season 1 and season 2 are presented in the Table 7.

The results indicated that in season 1, application of inorganic fertilizers and biofertilizers had significant influence on Essential oil content (%). Among the treatments, T_2 (100% RDF + *Azospirillum*) recorded significantly maximum essential oil content (0.91 %) which was statistically on par with T_1 (100% RDF + *Azotobacter*),

T_6 (75% RDF + *Azospirillum*) and T_5 (75% RDF + *Azotobacter*), while minimum value (0.75%) was recorded in T_{11} (50% RDF + *VAM*).

All treatments differed significantly with respect to essential oil content (%) during season 2. Among the treatments, T_2 (100% RDF + *Azospirillum*) recorded significantly maximum essential oil content (%) (0.83 %) which was statistically on par with T_1 (100% RDF + *Azotobacter*), T_6 (75% RDF + *Azospirillum*) and T_5 (75% RDF + *Azotobacter*), while minimum value (0.66 %) was recorded in T_{11} (50% RDF + *VAM*).

Essential oil content and its yield is an important consideration in any aromatic crop in realizing the maximum returns. The essential oil content in Japanese mint was significantly influenced by the application of inorganic fertilizers and biofertilizers in season 1 and season 2. In the present study, the plant supplied with T_2 (100% RDF + *Azospirillum*) recorded significantly the highest essential oil content compared to T_{11} (50% RDF + *VAM*) which recorded the least percentage of oil. This was due to the application of biofertilizers which helped in promoting thenutrient availability which inturn increased the essential oil to a greater extent. These findings were in agreement with the findings of Khandeel *et al*. (2002) in *Ocimum basillicum*andAswani *et al*. (2020) in Japanese mint.

Table 7. **Effect of inorganic fertilizers and biofertilizers on fresh herb yield (q/ha), essential oil content (%), oil yield (kg/ha) of Japanese mint (*Menthaarvensis* L.) var. Kosi during Feb-May and Aug-Nov 2022.**

Treatments	Fresh herb yield (q/ha)		Essential oil content (%)		Oil yield (kg/ha)	
	Feb-May 2022	Aug-Nov 2022	Feb-May 2022	Aug-Nov 2022	Feb-May 2022	Aug-Nov 2022
T_1: 100% RDF+Azotobacter	224.84	219.58	0.90	0.82	202.80	180.49
T_2 : 100% RDF + Azospirillum	225.04	221.34	0.91	0.83	205.69	184.15
T_3 : 100% RDF + VAM	190.35	189.39	0.84	0.76	159.89	144.12
T_4 : 100% RDF + PSB	200.67	191.86	0.85	0.77	170.57	148.28
T_5 : 75% RDF + Azotobacter	220.79	213.20	0.88	0.81	195.18	175.10
T_6 : 75% RDF + Azospirillum	222.14	216.41	0.89	0.82	197.70	176.66
T_7 : 75% RDF + VAM	176.17	175.20	0.83	0.74	145.69	129.48
T_8 : 75% RDF + PSB	172.05	171.90	0.83	0.75	143.32	128.07
T_9 : 50% RDF + Azotobacter	153.98	150.05	0.81	0.72	124.26	107.74
T_{10} : 50% RDF + Azospirillum	148.99	145.76	0.79	0.71	118.15	102.76
T_{11} : 50% RDF + VAM	144.08	139.78	0.75	0.67	109.31	93.75
T_{12} : 50% RDF + PSB	147.34	142.45	0.76	0.66	110.07	93.87
T_{13} : Control (RDF-160:50:40 NPK kg/ha)	182.52	180.96	0.83	0.75	150.88	135.48
SEm±	4.10	3.45	0.018	0.022	4.44	4.16
CD @ 5%	11.98	10.08	0.051	0.064	12.96	12.14

3.1.9. Essential oil yield (kg/ha)

Data with regard to the effect of inorganic fertilizers and biofertilizers on essential oil yield (kg/ha) is presented in Table 7.

The results indicated that in season 1 application of combination of inorganic fertilizers and biofertilizers had significant influence on essential oil yield. Among the treatments, T_2 (100% RDF + *Azospirillum*) recorded significantly maximum essential oil yield (205.69 kg) which was statistically on par with T_1 (100% RDF + *Azotobacter*), T_6 (75% RDF + *Azospirillum* and T_5 (75% RDF + Azotobacter), while minimum value (109.31 kg) was recorded in T_{11} (50% RDF + *VAM*).

All treatments differed significantly with respect to essential oil yield (kg/ha). Among the treatments, T_2 (100% RDF + *Azospirillum*) recorded significantly maximum essential oil yield (184.15 kg) which was statistically on par with T_1 (100% RDF + *Azotobacter*), T_6 (75% RDF + *Azospirillum*) and T_5 (75% RDF + *Azotobacter*), while minimum value (93.75 kg) was recorded in T_{11} (50% RDF + *VAM*) during season 2.

Azospirillium 20g /kg had significant influence on growth and quality attributes of the plant, this might be due to the increased uptake of available major nutrients to the plant which results in the translocation of nutrients to the plant parts. It can fix large amount of nitrogen which is freely available at atmosphere and also involved in protein synthesis, increased activity of Gibberellic acid, indole acetic acid and dehydrogenase activity, which might have improved the growth characters like plant height, leaf length, leaf area and ascorbic acid content in leaves. These results are in accordance with the findings of Jadhav (2014).Oil production is the most important parameter in Japanese mint farming. Integrated nutrient management improve the chemical, physical and biological soil proprieties that reflect positively on plant growth and oil yield (Patra *et al.* 2000). These results are in conformity with cheena *et al.*(2020) in Ocimum gratissimum.

3.1.10 Menthol content

It is clear from Table 8 that menthol content did not vary significantly among different treatments during season 1 and season 2.

Table 8 Effect of inorganic fertilizers and biofertilizers on menthol content (%) of Japanese mint (*Menthaarvensis* L.) var. Kosi during Feb-May and Aug-Nov 2022.

Treatments	**Menthol content (%)**	
	Feb-May 2022	**Aug-Nov 2022**
T_1: 100% RDF+Azotobacter	76.60	75.93
T_2 : 100% RDF + Azospirillum	76.56	75.90
T_3 : 100% RDF + VAM	76.13	75.47

T_4 : 100% RDF + PSB	76.53	75.87
T_5 : 75% RDF + Azotobacter	76.33	75.67
T_6 : 75% RDF + Azospirillum	76.07	75.40
T_7 : 75% RDF + VAM	75.83	75.17
T_8 : 75% RDF + PSB	76.30	75.63
T_9 : 50% RDF + Azotobacter	76.43	75.76
T_{10} : 50% RDF + Azospirillum	75.80	75.13
T_{11} : 50% RDF + VAM	76.30	75.63
T_{12} : 50% RDF + PSB	76.07	75.40
T_{13} : Control (RDF-160:50:40 NPK kg/ha)	76.37	75.70
SEm±	0.43	0.45
CD @ 5%	**NS**	**NS**

4. **CONCLUSION:**From the study it was concluded that we can decrease the inorganic fertilizer usage upto 25 % by adding azospirillum or azototobacter to get the optimum yield and benefit cost ratio in Japanese mint.Bio-fertilizers are known to be ecofriendly as well as environmentally safe. They are available at low cost which is of immense help to the farmers. Also, biofertilzers have positive alternative to chemical one. They are very safe for humans and animals, and reduce pollution of environment. Use of biofertilizer enhances growth and yield promoting parameters.As in this study pronounced yield was obtained by the application of biofertilizers withreduced dose of inorganic fertillizers. Overall, utilization of biofertilizers in combination with reduced dose of inorganic fertilizers to increase in yield could be a strategy to achieve sustainable production in Japanese mint in near future.
5. **Disclaimer (Artificial intelligence)**
6. Option 1:
7. Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.
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10. Details of the AI usage are given below:
11. 1.
12. 2.
13. 3.
14.

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