Original Article

The Effect of Fertilization and *Mycorrhiza* Inoculation on Yield Variables and Essential Oil Characteristics of *Salvia officinalis* L. Growing in the Greenhouse and at the Field

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ABSTRACT

Common sage (*Salvia officinalis* L.) is one of the most valuable commercial medicinal and aromatic plants (MAP). It has served different treatments historically in folk medicine and has always been popular to use. This research was conducted to observe the effect of mycorrhiza infection and fertilization to the biomass and quality characteristics on common sage. The optimum N, P, K dosses and their combinations (NP, NK, PK, NPK) were applied to both +M (mycorrhiza infected) and –M (non mycorrhiza infected) plants. The research was carried out both in the greenhouse and the plants were transferred to the field. The field trial was established in randomized block design with tree replication at ALC, UMass and three harvests were recorded at the growing season in 2014. Yield parameters showed significance between –M and +M applications. The essential oil contents were extracted by vapor distillation, and the major components of the essential oils were determined as camphor, α-thujon, ß-thujon, α-humulene, viridiflorol and eucalyptol (1.8 cineole) by GC-Ms. The highest camphor was received from P (-M) and PK (+M) fertilization with 31.64% and 33.54%. And the highest α-thujon was recorded at PK (-M) and NK (+M) combinations with 27.51% and 34.24%, respectively.

Keywords: Common Sage (*salvia officinalis* L.), Fertilization, *Mycorrhiza* Spp. Biomass, Essential Oil Yield, Camphor, Α-Thujone.

INTRODUCTION

Salvia officinalis, *Lamiaceae*, known as Dalmatian Sage has been gaining popularity in every industrial sector. Wide adaptability and non-selective climatic requirements made it possible to receive high biomass and several harvests during the same plantation period. Current uses of sage include the following: indigestion, treatment of inflammation of the mouth and throat, excessive sweating, including that associated with peri-menopause; relief of pressure spots that result from the use of a prosthesis; and as a flavoring for food. Sage oil has also been employed as a fragrance in soaps and perfumes.[5-10-14-18,19] Due to global warming, more stress resistant, and drought and nutrient tolerant plant production is needed in the landscape. Therefore, the use of mycorrhiza fungi in plant production has gained importance. They are even acting to promote uptake and enhance the biomass.

The benefits of mycorrhiza can be masked by cultural conditions.[21-24,25,26] The benefits are also detectable when the plants are exposed to stress conditions but they may not be applicable for all production systems or landscape situations. Certain cultivars can be more responsive to specific fungi while others may be more sensitive to fertilizer types.[3] Mycorrhizal colonization frequency may change under different levels of soil fertility that may alter the net costs and

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benefits received by plant hosts.[21-30,31,32] Fertilization may decrease or eliminate the net benefits that mycorrhizae provide to infected plants (manifested as increased growth and survival) because the plant's cost of carbon allocation to the fungus may not be offset by the benefits of hosting fungi when soil nutrients are less limiting to the plant.[29-31] The present study was conducted both in the greenhouse (University of Massachusetts, Stockbridge Agriculture, CNS), and in the field (Agricultural Learning Center, UMass, Amherst, MA) in order to investigate the effect of mycorrhiza and various fertilization doses to the biomass, and essential oil characteristics of common sage.

EXPERIMENTAL MATERIAL AND METHODS

Common sage plants (*Salvia officinalis* L.) from Burpee Garden Products Co. were grown from seeds in the CNS Research & Education Greenhouse for a 12 h photoperiod in 10th February 2014. The day/night temperature was 22/15°C. Sun Gro LC1 growing mix (formulated with Canadian sphagnum, peat moss, coarse pearlite, starter nutrient charge with gypsum, and dolomite limestone), was used as the growth medium throughout the course of the Greenhouse experiment. The seeds were sown in LC1 growing mix filled seed trays. 6 weeks after germination, the plants reached an optimum size of about 4-6 cm and were transplanted into 6 inch (15.24 cm) pots. A granular mixture of mycorrhiza fungus *Glomus aggregatum*, *G. intraradices*, *G. mossae*, *G. etunicatum*, *G. monosporum*, *G. deserticola* and *Glomus clarum* and some bacteria strains (*B. licheniformis, B. azotoformans, B. megaterium, B. congulans, B. pumilis, B. polymyxa, Sacromyces cervisiae, Streptomyces griseous, S. lyndicus, Pseudomonas aurofaceans, P. fluorescence,* 50.000 cfu) and trichoderma granular spores (*T. koningii* and *T. harzianum*; 187.500 spores) from Myco Maximum, Humboldt Nutrients were applied first to the trays than to the pots as a suggested dose of 4 oz per 1.5-3 cu. F/t. of media. Nitrogen, phosphorus and potassium and their combinations (Control, N, P, K, NP, NK, PK and NPK) were applied both mycorrhiza infected and non-infected samples. N:P2O5:K2O was applied with 1:0.43:0.56 dosses to the pots.[39]

The study was carried out both in the Greenhouse and afterwards at the experimental field of the Agricultural Learning Center at the University of Massachusetts. The trial was established as a randomized block design with three replicates. Following observations were taken, one from the greenhouse (May 2nd, 2014) and two from field experiments (July 31st and October 8th, 2014), in total, from three harvests as; plant height (cm), number of shoot, canopy width (cm), 90° canopy width (cm), fresh herb (g), dry herb (g), and leaf area index (cm^2). Essential oils were extracted by steam distillation apparatus in the laboratory of Medicinal and Aromatic Plants Program, UMass, Amherst. Essential oils were characterized by GC-MS in the Medicinal and Aromatic Plants Laboratory of Bati Akdeniz Agricultural Research Institute.

The plant samples for essential oil analyses were taken from 9 plants representing each application. Fresh material was kept to dry 3 days in a 35°C incubator, inside paper lunch bags. Dry leaves (100 g) were placed in a distillation apparatus with 2 L of distilled water and vapor distilled for 3 h. Steam distillation of MAPs were recommended by several previous studies for lavender and rosemary.[35-6-8] The GC/MS analysis was carried out with an Agilent 5975 GC-MS system. Innowax FSC column (60m x 0.25mm, 0.25µm film thickness) was used with helium as carrier gas (0.8 mL/min.). The samples were diluted with hexane 1:100 and were injected into the column (0.2 µl) with a split ratio of 40:1. The initial oven temperature of the column was 60°C and was raised to 220°C with a rate of 4°C/min and then kept constant at 220°C for 10 min. The injector temperature was at 250°C. The total analyses duration was 60 min for each sample. Scanning range for the mass detector was m/z 35 to 450 and 70 eV electron bombardment ionization was used. The components of essential oils were identified by comparison of their mass spectra with those in the Adams Library, Wiley GC/MS Library, Mass Finder Library, and confirmed by comparison of their retention indices (RRI). The results were analyzed by analyses of variance and ranged by Duncan's multiple range tests.[1-13-27-16]

Three soil samples were taken from the experimental field on July 22, 2014. Soil nutrient analyses were conducted in the Soil and Plant Tissue Testing Laboratory, West Experiment Station of University of Massachusetts, Amherst on July 25, 2014. The mean of P (phosphorus) value provided from experimental field was 21.3 ppm (4-14 ppm optimum dosses) and K (potassium) 284.3 ppm (100-160 ppm). These amounts were defined as above optimum. Only the Ca (calcium) amount of the soil was found lower with 557ppm (1000-1500 ppm).

RESULTS AND DİSCUSSİONS

Arbuscular mycorrhizal fungi are known to play an important role in plant nutrition and biomass production in many agricultural systems. Although, it is known limited about their potential effect on secondary metabolites in medicinal and aromatic plants.[11-22,23] AMF symbiosis may improve nutrient uptake by improving the soil exploration and contributes to enhance the growth and vigor of plants.[7] These interactions may be of crucial importance within sustainable, low-input agricultural cropping systems that rely on biological processes rather than agrochemicals to maintain soil fertility and plant health.[4]

The greenhouse experiment revealed that myco inoculated pots were recorded high fresh, dry herb and a high leaf area index considered to non myco inoculated plants as shown in Table 1. All the applications were found at the level of 1% to be statistically significant. The highest plant height was obtained from NK application with an average of 21.49 cm from –M application in greenhouse production. The plant height changed from 18.7cm to 26.87 cm at +M applications. NPK (26.87 cm), NP (26.17 cm) and N (25.24 cm), located among the same statistical group, giving the highest plant height at +M as shown at Table 1. Nitrogenous fertilization increased the plant height in both –M and +M applications. The mean number of shoots was 13.70 (-M) and 13.92 (+M). Although sage tends to grow upright, various applications or stress factors may let it grow horizontally. The mean canopy width ranged from 22.80 cm to 24.07 cm, and from 19.23 cm to 22.06 cm respectively, measuring 90 degrees angles. The mean dry herb yield was found to be 1.82 g (-M) and 2.33 g (+M) per plant. The highest dry herb yield for –M was obtained from 2.79 g (N), 2.75 g (NK), 2.72g (NP) and 2.65 g (NPK) applications. The highest dry weight for +M ranged between 3.50 g (N) and 3.23 g (NPK). The mean leaf area index of greenhouse harvesting for –M was 329.57 cm² and for +M, 418.98 cm². As seen from Table 2. myco application enlarged the leaf area and enhanced the biomass yield.

All plants from the pots were transferred to the field of ALC at UMass in early June (June 2nd, 2015), by randomized block design technique with three replicate. There were three plants in each replicate, so nine plants were evaluated in each application.

The mean plant height for the field harvest was 29.40 cm and 29.42 cm in both parcels, -M and +M, respectively. Although there was no significant difference regarding plant height and number of shoots, the fresh and the dry herb yield of –M and +M applicated plants were found statistically significant at the level of 1% as seen at Table 3.

Myco inoculation from field experiments; both from the second (May 6th, 2014) and from the third yield harvests (October 8th, 2014), showed the promotion of yield and leaf area indexes of sage production. Except plant height and essential oil yield, all yield parameters were shown statistical significance at a level of 1% in the second harvest as seen at Table 4. The mean fresh

Table 1: The mean yield parameters of *Salvia officinalis* at pot experiments from –M (none mycorrhiza application) and +M (mycorrhiza application) in greenhouse. Plant height (cm), Number of shoot, Canopy width (cm), Canopy width 90° (cm), fresh herb (g), dry herb (g), and Leaf area index cm² from the first harvest, May 6th 2014.

	Plant Heigth		Number of Shoot		Canopy Width		Canopy Width 90°		Fresh Herb		Dry Herb		Leaf Area index (cm²)	
	-M	+M	-M	+M	-M	+M	-M	+M	-M	+M	-M	+M	-M	+M
Control	19.66ab	18.7b	12.56c	13.00bc	17.76c	21.68cd	16.76c	20.46d	6.47b	9.77cd	1.28b	1.92cd	196.05b	248.49cd
N	20.78ab	25.24a	16.67a	16.56a	30.26b	31.50a	26.42a	28.74a	17.62a	22.66a	2.79a	3.50a	506.61a	667.88a
P	11.55c	18.18b	11.44c	10.67d	13.54c	17.49e	11.92d	16.17e	8.37b	4.98e	0.53c	1.02e	68.124c	160.99d
K	11.76c	19.49b	11.11c	12.00cd	13.04c	20.42de	12.01d	18.08de	2.38b	6.76de	0.58c	1.32de	70.022c	206.94d
NP	19.71ab	26.17a	16.00ab	14.78ab	38.04a	25.18bc	25.70a	21.11d	17.99a	17.38b	2.72a	2.56bc	480.91a	522.65b
NK	21.49a	19.81b	14.67b	15.67a	26.31b	26.49b	22.27b	25.03bc	17.78a	20.94ab	2.75a	3.05ab	536.03a	604.73ab
PK	19.28b	19.52b	12.22c	12.67bcd	17.79c	21.46cd	15.62c	21.44cd	6.54b	11.72c	1.34b	2.09c	191.23b	350.3c
NPK	20.57ab	26.87a	15.00ab	16.00a	25.71b	28.34ab	24.11ab	25.44ab	18.82a	20.97ab	2.65a	3.23a	587.67a	589.77ab
Mean	18.07	21.75	13.70	13.92	22.80	24.07	19.35	22.06	11.99	14.4	1.82	2.33	329.57	418.97
P	**	**	**	**	**	**	**	**	**	**	**	**	**	**
LSD	2.15	3.43	1.98	2.30	7.71	3.78	3.22	3.67	7.63	4.12	0.60	0.66	109.05	120.50

* : 1%, ** : 5% statistically significant

Table 2: The mean yield parameters of *Salvia officinalis* at field trial from –M (none mycorrhiza application) and +M (mycorrhiza application) at ALC, UMass, Amherst. Plant height (cm), Number of shoot, Canopy width (cm), Canopy width 90° (cm), fresh herb (g), dry herb (g), essential oil yield (%) and Leaf area index cm^2 from second harvest (July the 31st, 2014).

	Plant Heigth		Number of Shoot		Canopy Width		Canopy width 90°		Fresh Herb		Dry Herb		Fresh Leaf		Dry Leaf		Essential Oil		Leaf Area index	
	-M	+M	-M	+M	-M	+M	-M	+M	-M	+M	-M	+M	-M	+M	-M	+M	-M	+M	-M	+M
Control	32.01	28.67b	17.67bc	12.11c	56.22bcd	58.78d	73.89b	55.67b	225.56bc	259.33c	49.67bcd	56.33c	169.89bc	185.11d	39.00bcd	43.22c	1.20b	1.26c	3473.30b	3732.64d
N	30.33	28.63b	23.56a	24.22a	67.33a	73.32a	66.33a	68.59a	392.56a	492.09a	90.22a	107.50a	296.67a	366.48a	70.56a	85.10a	1.40ab	1.60a	5397.30a	7031.92a
P	27.89	27.89b	12.22d	12.11c	51.11d	50.11e	46.78cd	48.44c	162.22cd	162.00d	34.33de	38.00d	129.44cd	121.67e	28.44de	30.56c	1.58a	1.25c	2596.56bc	2515.19e
K	26.56	27.78b	11.89d	12.22c	42.44e	51.11e	39.56d	46.44c	100.56d	181.11cd	22.44e	39.44cd	80.556d	140.67de	18.56e	32.00c	1.20b	1.30bc	1638.89c	2760.62de
NP	27.11	30.96ab	24.33a	17.43b	59.67abc	68.9ab	53.78bc	67.98a	250.33b	448.16ab	53.67bc	94.56ab	194.44b	333.47ab	43.11bc	73.23ab	1.60a	1.43abc	3508.28b	6565.59ab
NK	27.56	29.07b	21.31ab	17.7b	52.51cd	66.23bc	47.24cd	62.48a	198.51bc	376.72b	43.69cd	85.03b	156.77bc	284.74bc	36.03cd	66.09b	1.53a	1.30bc	3189.28b	5558.47bc
PK	33.02	33.11a	18.11bc	10.89c	63.00ab	62.56cd	59.89ab	67.78a	347.56a	374.78b	63.00b	87.56b	268.44a	269.22c	50.67b	66.00b	1.13b	1.46ab	5005.71a	5049.64c
NPK	30.77	29.30b	14.71cd	22.32a	60.82ab	66.7bc	66.22a	66.05a	390.39a	442.93ab	92.47a	100.00ab	308.91a	341.96ab	71.43a	77.66ab	1.50a	1.46ab	5226.81a	6442.90ab
Mean	29.40	29.42	17.97	16.1	56.63	62.25	54.67	60.47	258.41	342.66	56.18	76.25	200.60	255. 80	44.71	59.37	1.39	1.38	3754.36	4963.31
P	NS	*	**	**	**	**	**	**	**	**	**	**	**	**	**	**	*	*	**	**
LSD	4.70	3.16	3.84	3.30	7.84	5.79	7.77	6.52	71.00	79.78	15.39	17.76	54.91	55.74	11.93	13.47	0.28	0.19	1031.06	1115.10

* : 1% ,** : 5% statistically significant NS: Not Significant

Table 3: The mean yield parameters of *Salvia officinalis* at field trial from –M (none mycorrhiza application) and +M (mycorrhiza application) at ALC, UMass, Amherst. Plant height (cm), Number of shoot, Canopy width (cm), Canopy width 90° (cm), fresh herb (g), dry herb (g) from third harvest (October the 8th, 2014).

	Plant Heigth		Number of Shoot		Canopy Width		Canopy width 90°		Fresh Herb		Dry Herb		Dry Leaf	
	-M	+M	-M	+M	-M	+M	-M	+M	-M	+M	-M	+M	-M	+M
Control	19,97ab	23,33	54,74bc	49,44bc	53,75a	53,56a	52,02ab	52,78ab	202,53a	220,78ab	45,15a	52,5ab	33,43a	37,34abc
N	19,33abc	20,78	61,64ab	58,44ab	54,64a	57,22a	53,78ab	56,67a	195,06a	260,56a	44,66a	59,79a	35,77a	45,14a
P	18,11bcd	19,56	32.00d	42,11cd	35,78cd	51.00ab	37,44d	52,56ab	95,22bc	215,89abc	22,89cd	49,32abc	16,03cd	37,88abc
K	16,57d	21.00	29,33d	35,56d	33,09d	49,44ab	34,05d	47,67bcd	76,58c	166,11bc	17,43d	37,14bcd	13,41d	27,53cd
NP	18,06bcd	18,78	42,93c	40,78cd	42,12bc	44.00b	40,12cd	43,89d	120,84b	152,78c	26,11cd	32,55d	20,68bc	24,99d
NK	17,45cd	18,20	36,21d	43,09c	39,01cd	41,89b	36,9d	42,54cd	90,427bc	148,98bc	18,84d	31,56cd	15,06d	24,81bcd
PK	21,67a	22,22	55,44bc	59,89a	51.00ab	56,67a	46,56bc	53,67ab	126,22b	219,11ab	28,93bc	50,41abc	21,72c	39,36ab
NPK	20,30ab	20,69	65,49a	60,65a	49,22ab	50,74ab	52,51a	52,19abc	158,58a	190,79bc	34,81ab	44,99abcd	26,2ab	35,37abcd
Mean	18.80	20.57	48.04	49.12	45.22	50.67	44.46	50.43	135.77	198.24	30.26	45.18	23.20	34.45
P	*	NS	**	**	**	*	**	*	**	*	**	*	**	*
LSD	2.81	3.15	10.94	10,43	8.7	9.17	8.15	8.05	49.63	65.65	11.23	15.62	8.12	11.54

*: 1% ,** : 5% statistically significant NS: Not Significant

Table 4: The main (camphor, α-thujone, β-thujone, α-humulene, viridiflorol, eucalyptol, camphene and α-pinene) essential oil components of *Salvia officinalis* plantation from second (field) harvest (July the 31st, 2014).

	Camphor		α-thujone		β-thujone		α-humulene		*Viridiflorol*		*Eucalyptol* (1.8 cineole)		*camphene*		α-pinene	
	-M	+M	-M	+M	-M	+M	-M	+M	-M	+M	-M	+M	-M	+M	-M	+M
Control	27.36	30.32	19.11	22.26	10.21	6.60	7,85	8,15	7.05	5.94	7.49	5.59	3.10	4.05	2.36	3.51
N	25.53	28.75	23.74	25.76	**23.74**	6.12	**8,30**	6,07	5.70	5.46	8.09	6.63	2.54	3.29	3.64	2.75
P	**31.64**	30.32	25.22	19.90	5.27	**10.51**	7,70	**7,58**	5.43	5.55	6.92	7.76	3.64	3.51	1.37	1.94
K	24.99	29.52	23.28	21.30	8.90	11.17	6,14	6,26	**8.10**	4.87	6.52	4.80	3.73	3.84	2.65	1.42
NP	29.39	20.94	23.55	29.00	10.16	6.27	6,37	6,84	3.46	4.70	6.04	7.50	3.56	4.01	1.36	7.25
NK	29.33	23.38	14.68	**34.24**	8.89	6.16	5,80	6,00	5.89	5.14	**9.15**	6.30	**4.56**	3.27	**3.78**	2.26
PK	24.14	**33.54**	**27.51**	19.25	8.62	4.86	7,43	6,77	5.87	6.07	5.26	6.82	2.02	**4.78**	1.63	1.80
NPK	30.25	15.91	24.00	25.33	5.01	4.26	7,62	6,75	5.12	**6.29**	6.46	**11.27**	3.80	4.51	1.49	**9.87**

herb, dry herb yield, fresh leaf, dry leaf and leaf area indexes were recorded at non-mycorrhiza infected (-M) and mycorrhiza infected (+M) plants as; 258.41g and 342.66g; 56.18 g and 76.25 g; 200.60g and 255.80g; 44.71g and 59.37g; 3754.36 cm^2 and 4963.31 cm^2 per plant, respectively. N fertilization both in myco (+M) and non-myco (-M) application gave the highest fresh and dry leaf parameters and leaf area indexes. The common point of all cuttings revealed that myco application gave positive interaction with fertilizer doses. Especially with Nitrogen and its combinations. Nitrogen fertilization enhanced the essential oil yield in both treatments and among their applications. The highest essential oil yield was obtained with 1.60% from –M NP combination and 1.60% from +M N combination. The mean essential oil yield was found to be 1.38% (-M) and 1.39% (+M). Although no significant differences found between the treatments –M and +M, effect of fertilizer applications was significant at the 5% level.

Camphor is today mostly used in the form of inhalants and of camphorated oil, a preparation of 19% or 20% camphor in a carrier oil, for the home treatment of colds[28] and as a major active ingredient of liniments and balms used as topical analgesics.[38] It is familiar to many people as a principal ingredient in topical home remedies for a wide range of symptoms, and its use is well consolidated among the population of the whole world, having a long tradition of use as antiseptic, antipruritic, rubefacient, abortifacient, aphrodisiac, contraceptive and lactation suppressant.[40] In the present study, the highest quantities of camphor was recorded at P (-M) and PK (+M) combinations as 31.64% and 33.54% respectively. These amounts were extremely high considered previous studies isolated from essential oil.[9-42] The reason why the camphor ratio was found to be so high might be due to the first year of the plantation. According to[12] the camphor content of sage leaves increases when the leaves enlarge. This increase is roughly proportional to the number of filled peltate oil glands which appear on the leaf surface during the expansion process. This, supports that immature sage leaves synthesize and accumulate camphor most rapidly. A-thujone found the other main constituent of the essential oil. In the United States, the addition of pure thujone to foods is not permitted.[41] Sage and sage oil (which can be up to 50% thujone) are on the Food and Drug Administration's list of generally recognized as safe (GRAS) substances.[2] The highest α-thujone was recorded at PK (-M) and NK (+M) combinations with 27.51% and 34.24%, respectively. Although this amount seems a high percentage, it was under the limit of given by the FDA and ISO 9909. But, according to

Figure 1: a. b. c. Images of *Salvia officinalis* during pot experiments at CNS (The College of Natural Sciences) Greenhouse (a,b) and Leaf Area Index (LAI) measurements, UMass, Amherst.

Figure 2: d,e and f. Establishing the field and transplanting the plants to the field (d,e), recording the observations (f) at ALC (Agricultural Learning Center), UMass, Amherst.

the German Drug Codex the thujones should be equal or more than 20.0% and the camphor should be around 4.5-24.5%. ISO 9909 for medicinal uses regulates the amounts of the constituents in the sage essential oils for camphor 4.5-24.5% and α-thujone 18.0-43.0%.[33,34-36]

CONCLUSION

Myco application gave positive interaction with fertilizer doses, especially with Nitrogen and its combinations both in the greenhouse and at the field conditions. N fertilization promote the biomass and enhanced the essential oil yield in both treatments (-M and +M) and among their combinations.

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CONFLICT OF INTEREST

None

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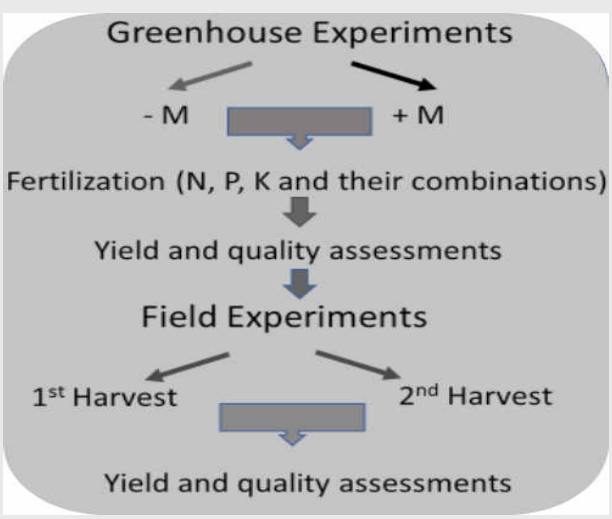
PICTORIAL ABSTRACT



SUMMARY

- Arbuscular mycorrhizal fungi and different ferti -lizer application on common sage production were investigated both in the greenhouse and at the field.
- Myco application gave positive interaction with fertilizer doses, especially with Nitrogen and its combinations.
- The mean essential oil yield was found to be 1.38% (-M) and 1.39% (+M). Although no significant differences found between the treatments −M and +M.
- The highest quantities of camphor was recorded at P (-M) and PK (+M) combinations as 31.64% and 33.54% respectively.

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