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Physiological attributes and essential oil production in *Mentha arvensis* L. treated with degraded oligomers of sodium alginate under field conditions

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Abstract
Menthol, obtained from essential oil of Japanese mint (*Mentha arvensis* L.) is used in pharmaceutical and cosmetic industries. Polysaccharides extracted from seaweeds showed effective biological activities in the degraded form. In the present study, the growth-enhancing character of radiation-processed sodium alginate (RPSA) was studied on biosynthesis and quality of essential oil production in commercially significant cash crop Japanese Mint. Under field conditions, two experiments were carried out in two successive years, to study the effect of radiation-processed sodium alginate on the overall performance of the crop. Sampling was done with maintaining uniform break of 15 days at 120, 135 and 150 days after sowing (DAS). Most of the parameters studied were found significantly affected by various concentrations of RPSA treatment. However, a non-significant trend was found for specific gravity and refractive index of mentha oil extracted from RPSA-treated plants. Treatments of RPSA had no effect on menthol content at 120 DAS, while it was proved effective to increase overall production of menthol over the control plants.

Keywords: radiation-processed polysaccharides, menthol, sodium alginate, essential oil, Japanese mint

Introduction
Crop, whose secondary metabolites are valued for their distinguished aromatic or therapeutic properties are more worth trading than the traditional food, forage or fiber crops [1]. Flavoring agents, fragrances, insecticides, dyes, and drugs are the ultimate products of secondary metabolites found in medicinal and aromatic plants. The growth rate of essential oil trade usually is of 9% and 25% for local and export market, respectively. This force is worth attracting the attention of plant scientists to increase the essential oil production of these plants. Japanese mint is an important source of essential oil called mentha oil. The oil is used in several pharmaceutical formulations and flavor industries [2]. The oil is used to extract menthol, a waxy and crystalline substance that is extensively used to ease slight throat irritations and sunburns due to its cooling properties [2]. The demand for menthol is in great context and as it was assessed that the worldwide use of menthol was about 30–32,000 metric tonnes per annum in recent years [3]. Globally, roughly 10,000 tonnes of pure menthol are used by the pharma, cosmetic and other foods industries excluding 2,000 tonnes of synthetic menthol [3]. India export about 25 to 30,000 tons in various shipping arrangement (as menthol crystals, powder, dementholised mint oil, etc.) as well as equal production of mint is used by domestic industries. India domestic intake is around 40% of globally trade, with China (20%), Europe (15%, with Germany and Netherlands the major users) and the USA (15%) accounting for the bulk of consumption [4]. Recently, plant scientists used oligomers of seaweeds to augment the output of various medicinal plants by using radiation technique. Irradiation with cobalt-60 degrades the natural polysaccharides into oligomers with low molecular weight. It is well known that application of the degraded natural polysaccharides of sodium alginate, chitosan and carrageenan (oligomers) through leaf stimulate a variety of activities in plants such as growth, metabolism, cell division etc. [5-12]

Sodium alginate is one of the most available polysaccharides in nature. It is obtained mainly from brown marine algae and some species of bacteria [13]. Various researchers have reported the significant promotive effect of radiation processed sodium alginate and other degraded polysaccharides on plant growth and developments [7-11], [14-18]. Keeping in mind the amazing properties and high demand of essential oil of Japanese mint (*Mentha arvensis* L.) and effect of the degraded oligomers of sodium alginate on the crop, a question arises that 'Could we augment the foliage, oil production, and menthol by using radiation-processed sodium alginate?'

Materials and methods
Plant resources and growth environment
In two consecutive years, experiments were carried out on Japanese mint under randomized block design field layout to study the effect of RPSA @ 20, 40, 60, 80, 100, 120 mg L^{-1}. Vigorous rhizomes of mint were procured from (supplied by) the Central Institute of Medicinal and Aromatic Plants (CIMAP), Lucknow, UP (India). Four blocks, each of 4 m^2 were prepared in the field. Rhizomes of mint were implanted with a spacing of 45 × 45 cm. The chemical composition of soil was examined by the Government Soil-Testing Laboratory, Aligarh, India. Physico-chemical characteristics of the soil are represented as follow: Texture: sandy loam; pH (1:2): 7.4; E.C. (1:2): 0.49 m mhos cm^{-1}; nitrogen: 102.5 mg kg^{-1}soil, phosphorus: 7.9 mg kg^{-1} soil and potassium: 146.0 mg kg^{-1} soil.

Gel Permeation Chromatography (GPC) analysis

Radiation Chamber (BRIT, Mumbai, India) was used to irradiate samples by 520 kGy gamma radiation doses at a dose rate of 2.4 kGy/h. Hitachi Merck HPLC/GPC system was used to carry out GPC of the samples [15]. The various concentrations of irradiated sodium alginate (ISA) were finally prepared using double distilled water as spray treatments.

Growth and physiological parameters

The crop was assessed on the basis of physiological and yield characters at 120, 135 and 150 DAS. Five replicates of each treatment were analyzed. Chlorophyll and carotenoid contents were determined by the method of Lichtenthaler and Buschmann [19] using a spectrophotometer (Shimadzu UV-1700, Tokyo, Japan). Carbonic anhydrase (CA) activity (EC 4.2.1.1) was estimated by the method of Dwivedi and Randhawa [20]. Nitrate reductase (NR) activity (EC 1.6.6.1) was assessed by the method of Jaworski [21]. Contents of nitrogen (N), phosphorus (P) and potassium (K) in leaves were measured by the Kjeldahl method on a dry weight basis. The leaf-N content was determined by the method of Novozamsky *et al*. [22]. The method of Rorison *et al*. [23] was used to determine P content in leaf digested material. Potassium content in the leaves was analyzed flame-photometrically (Model, C150, AIMIL, India).

Yield and quality parameters

The extraction of essential oil from Mentha leaves was carried out by the method of Guenther [24]. Analysis of essential oil composition was done by GLC. The peaks obtained from GLC analysis compared with peaks of standard as cited by Adams [25]. The specific gravity of oil was measured by using a 'specific gravity bottle' as previously described. Refractive index of oil was estimated by the method of Jenkins and White [26]. The detailed procedure was given in the previous study.

Statistical analysis

Data were statistically analyzed through SPSS-17 statistical software (SPSS Inc., Chicago, IL, USA). Means were compared using Duncan's Multiple Range Test (DMRT) at $p \leq 0.05$.

Results

Significant differences observed among the various levels of RPSA application. However, application of RPSA @ 120 mg L^{-1} did not further enhance the values of parameters; however, significant differences were observed in comparison to control. The application of RPSA had a promotive effect on physiology (Table 1). The percent increase in chlorophyll content resulted from the application of RPSA @ 100 mg L^{-1} was 19.36, 22.73 and 25.01% over water sprayed control at 120, 135 and 150 DAS, respectively. An increase of 8.48, 11.77 and 11.30% was observed for total carotenoids content in RPSA @ 100 mg L^{-1}over the control at 120, 135 and 150 DAS, respectively. RPSA @ 100 mg L^{-1} significantly increased CA activity by 26.76, 28.32 and 35.02%, respectively over the control at 120, 135 and 150 DAS. RPSA @ 100 mg L^{-1} showed a significant increase in NR activity by 28.45, 32.99 and 35.25% at 120, 135 and 150 DAS, respectively over the control (Table 1). RPSA @ 100 mg L^{-1} proved to be the best and enhanced leaf-N content by 15.32, 18.40 and 24.05% over control at 120, 135 and 150 DAS, respectively. The application of RPSA @ 100 mg L^{-1} recorded 12.07, 12.16 and12.12% higher values for P content per plant at 120, 135 and 150 DAS, respectively, over control. RPSA @ 100 mg L^{-1} proved to be the optimum and enhanced leaf-K content by 8.97, 18.69 and 15.85% over control at 120, 135 and 150 DAS, respectively (Table 1).

Application of RPSA @ 100 mg L^{-1} enhanced essential oil content by 49.16, 54.91 and 56.52% over control at 120, 135 and 150 DAS, respectively. RPSA @ 100 mgL^{-1} enhanced essential oil yield by 152.08, 142.66 and 151.22% at 120, 135 and 150 DAS, respectively over control (Table 2). However, the effect of RPSA on specific gravity and refractive index of essential oil was found to be non-significant (Table 2). All the treatments of RPSA were found insignificant for menthol content at 120 DAS (Fig. 1). However, application of RPSA on menthol yield was found effective over control.

Table 1: Effect of six levels of radiation-processed sodium alginate (RPSA) on physiological attributes of *M. arvensis* at 120, 135 and 150 days after planting (DAP). (Data present mean of two years; means with same letters are not significantly different)

Parameters	DAP	Different levels of radiation-processed sodium alginate (mg L^{-1})						
		0	20	40	60	80	100	120
Total chlorophyll content (mg g^{-1} FW)	120	1.648 ± 0.005^{e}	1.703 ±0.005^{d}	1.735 ± 0.006^{c}	1.766 ± 0.004^{bc}	1.871 ± 0.008^{b}	1.967 ± 0.005^{a}	1.702 ±0.006^{bc}
	135	1.848 ± 0.006^{e}	1.909 ±0.007^{d}	1.956±0.005^{bc}	2.005 ± 0.006^{bc}	2.156 ± 0.004^{b}	2.268 ± 0.005^{a}	1.908 ± 0.004^{c}
	150	1.787 ±0.005^{e}	1.873 ±0.006^{d}	1.902 ± 0.008^{c}	1.966 ± 0.005^{bc}	2.046 ± 0.008^{b}	2.234 ± 0.005^{a}	1.883 ± 0.005^{c}
Total carotenoid content (mg g^{-1} FW)	120	0.590 ±0.004^{d}	0.593 ±0.002^{d}	0.607 ± 0.004^{c}	0.617 ± 0.006^{c}	0.625 ± 0.005^{b}	0.640 ± 0.008^{a}	0.631 ± 0.005^{b}
	135	0.620 ± 0.005^{e}	0.626 ±0.006^{d}	0.640 ± 0.005^{c}	0.648 ± 0.004^{b}	0.662 ± 0.006^{b}	0.693 ± 0.007^{a}	0.657 ± 0.005^{b}
	150	0.602 ± 0.002^{f}	0.607 ± 0.003^{e}	0.617 ±0.004^{d}	0.628 ± 0.005^{c}	0.639 ± 0.004^{c}	0.658 ± 0.006^{a}	0.670 ± 0.002^{b}
CA activity [µmol (CO_2) kg^{-1} (FW) S^{-1}]	120	230.9 ± 1.45^{f}	243.2 ± 1.25^{e}	250.0 ± 1.64^{d}	256.4 ± 2.05^{c}	270.7 ± 2.15^{b}	292.7 ± 2.31^{a}	254.4 ± 1.87^{c}
	135	247.3 ± 1.35^{f}	258.8 ± 1.28^{e}	268.2 ± 1.65^{d}	277.1 ± 1.58^{c}	309.7 ± 2.12^{ab}	317.3 ± 2.21^{a}	279.6 ± 1.18^{c}
	150	191.6 ± 1.14^{f}	195.5 ± 1.18^{e}	208.1 ± 1.64^{d}	226.8 ± 1.72^{c}	254.2 ± 2.08^{ab}	258.7 ± 1.82^{a}	228.7 ± 1.38^{c}
NR activity (nM NO_2^- g^{-1} FW h^{-1})	120	338.5 ± 1.85^{f}	362.6 ± 1.58^{e}	378.8 ± 1.72^{d}	397.8 ± 1.85^{c}	414.5 ± 1.69^{b}	434.8 ± 2.04^{a}	378.8 ± 1.48^{d}
	135	411.0 ± 1.75^{f}	447.4 ± 1.42^{e}	462.2 ± 1.68^{d}	479.4 ± 1.84^{c}	497.9 ± 1.71^{b}	546.6 ± 2.15^{a}	465.4 ± 1.49^{d}
	150	352.7 ± 1.82^{f}	389.0 ± 1.94^{e}	409.0 ± 2.02^{d}	422.1 ± 1.51^{c}	443.6 ± 1.48^{b}	477.0 ± 1.62^{a}	407.7 ± 1.52^{d}
Leaf N content (%)	120	2.48 ± 0.025^{e}	2.56 ± 0.028^{d}	2.57 ± 0.031^{d}	2.65 ± 0.025^{c}	2.69 ± 0.028^{b}	2.86 ± 0.028^{a}	2.54 ± 0.027^{d}
	135	2.50 ± 0.027^{e}	2.56 ± 0.028^{d}	2.57 ± 0.029^{d}	2.65 ± 0.027^{b}	2.69 ± 0.028^{b}	2.96 ± 0.025^{a}	2.54 ± 0.026^{cd}
	150	1.58 ± 0.22^{e}	1.70 ± 0.23^{d}	1.74 ± 0.24^{c}	1.75 ± 0.21^{c}	1.80 ± 0.28^{b}	1.96 ± 0.24^{a}	1.71 ± 0.26^{d}

Leaf P content (%)	120	0.232 ± 0.004^{f}	0.235 ± 0.005^{f}	0.241 ± 0.003^{e}	0.245 ± 0.002^{d}	0.249 ± 0.006^{b}	0.260 ± 0.004^{a}	0.252 ± 0.003^{c}
	135	0.222 ±0.005^{d}	0.225 ±0.004^{d}	0.231± 0.005^{c}	0.235 ± 0.004^{c}	0.242 ± 0.005^{ab}	0.249 ± 0.004^{a}	0.221 ± 0.006^{d}
	150	0.165 ± 0.007^{e}	0.168 ±0.004^{d}	0.170 ± 0.005^{c}	0.173 ± 0.003^{c}	0.176 ± 0.004^{b}	0.185 ± 0.004^{a}	0.169 ± 0.002^{c}
Leaf K content (%)	120	2.34 ± 0.013^{e}	2.36 ± 0.018^{d}	2.43 ±0.0.11^{cd}	2.47 ± 0.013^{c}	2.51 ± 0.014^{b}	2.55 ± 0.013^{a}	2.45 ± 0.014^{c}
	135	1.98 ± 0.014^{f}	2.16 ± 0.015^{e}	2.19 ± 0.018^{de}	2.24 ± 0.017^{c}	2.28 ± 0.016^{b}	2.35 ± 0.014^{a}	2.15 ± 0.012^{d}
	150	1.64 ± 0.012^{e}	1.70 ±0.016^{d}	1.73 ± 0.012^{c}	1.76 ± 0.012^{c}	1.79 ± 0.014^{b}	1.90 ± 0.012^{a}	1.65 ± 0.011^{d}

Table 2: Effect of six levels of radiation-processed sodium alginate (RPSA) on essential oil production in *M. arvensis* at 120, 135 and 150 days after planting (DAP). (Data present mean of two years; means with same letters are not significantly different)

Parameters	DAP	Different levels of radiation-processed sodium alginate (mg L^{-1})						
		0	20	40	60	80	100	120
Essential oil content (%)	120	0.657±0.005^{g}	0.703 ± 0.004^{f}	0.773±0.006^{e}	0.847 ± 0.004^{d}	0.960±0.004^{b}	0.980±0.005^{a}	0.887 ± 0.006^{c}
	135	0.967±0.006^{f}	1.027 ± 0.005^{e}	1.150±0.007^{d}	1.253 ± 0.005^{c}	1.473±0.005^{b}	1.498±0.004^{a}	1.283 ± 0.007^{c}
	150	0.867±0.006^{g}	0.933 ± 0.007^{f}	1.027±0.008^{e}	1.230 ± 0.006^{c}	1.313±0.007^{b}	1.357±0.005^{a}	1.213 ±0.004^{d}
Oil yield (L/ha)	120	134.4 ± 0.04^{g}	169.5 ± 0.06^{f}	203.5 ±0.08^{de}	254.6 ± 0.05^{c}	307.8 ± 0.03^{b}	338.8 ± 0.04^{a}	210.1 ± 0.05^{d}
	135	234.4 ± 0.05^{g}	279.7 ± 0.05^{f}	348.2 ± 0.08^{e}	427.7 ± 0.06^{c}	547.4 ± 0.04^{b}	568.8 ± 0.05^{a}	377.8 ± 0.04^{d}
	150	213.6 ± 0.05^{g}	261.1 ± 0.05^{f}	331.8 ± 0.05^{e}	437.4 ± 0.04^{c}	505.4 ± 0.05^{b}	536.6 ± 0.04^{a}	363.7 ± 0.05^{d}
Specific gravity of essential oil (g/cm^{3})	120	0.881±0.001^{a}	0.882 ± 0.002^{a}	0.883±0.003^{a}	0.882 ± 0.004^{a}	0.884±0.004^{a}	0.889±0.003^{a}	0.885 ± 0.004^{a}
	135	0.882±0.002^{a}	0.883± 0.003^{a}	0.884±0.004^{a}	0.884 ± 0.004^{a}	0.885±0.005^{a}	0.891±0.003^{a}	0.888 ± 0.004^{a}
	150	0.879±0.004^{a}	0.881± 0.005^{a}	0.881±0.003^{a}	0.882 ± 0.004^{a}	0.884±0.005^{a}	0.887±0.004^{a}	0.885 ±0.005^{a}
Refractive index of essential oil	120	1.451±0.001^{a}	1.453 ± 0.002^{a}	1.453±0.001^{a}	1.455 ±0.0002^{a}	1.459±0.002^{a}	1.462±0.003^{a}	1.458 ± 0.001^{a}
	135	1.456±0.002^{a}	1.457 ± 0.003^{a}	1.458±0.002^{a}	1.458 ± 0.004^{a}	1.460±0.004^{a}	1.464±0.005^{a}	1.457 ± 0.004^{a}
	150	1.453±0.001^{a}	1.455 ± 0.002^{a}	1.455±0.002^{a}	1.457 ± 0.002^{a}	1.459±0.002^{a}	1.461±0.002^{a}	1.458 ± 0.001^{a}

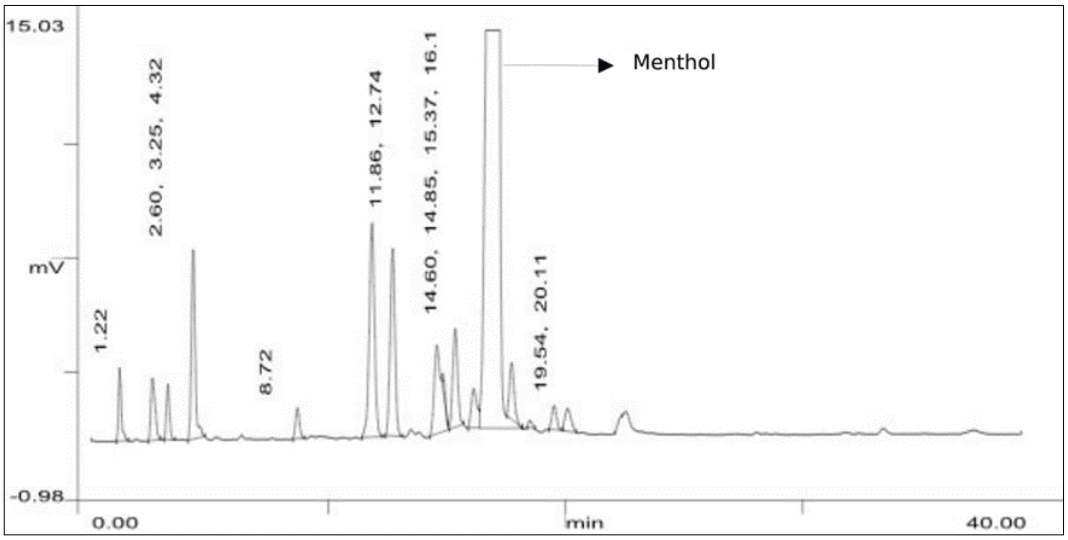

Fig 1: GC Chromatogram showing peaks of menthol content in control plants of *M. arvensis* L.

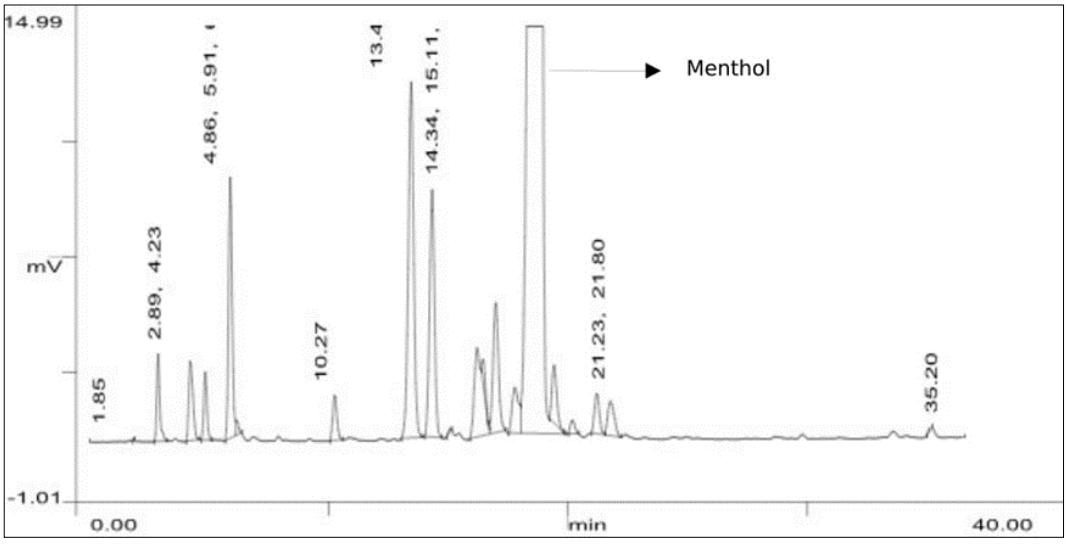

Fig 2: GC Chromatogram showing peaks of menthol content in radiation-processed sodium alginate (RPSA) treated plants of *M. arvensis* L.

Discussions

The irradiation of SA by gamma rays affects the overall polymer cross-linking process. As a result, its application influences the biological properties of the plant cells [27]. In addition, various researchers have accentuated that RPSA could productively act as a plant growth promoter and also a potent enhancer of the activity of various enzymes in the plants [28]. Plant growth regulators get involved through the modification of transcription, translation and/or differential sensitivity of the tissue. In the present investigation, RPSA was used as plant growth regulator. Growth attributes of mint were favorably stimulated by the foliar application of RPSA. The promoting effect of RPSA on growth parameters was more pronounced in the field experiment in comparison to pot experiment [29]. In field conditions, higher soil surface area was available which facilitated higher nutrient absorption by the roots [30]. Almost similar results were reported by various workers [8] for lemongrass, mentha, *Artemisia* and tomato seedling, respectively. It has been well established that exogenously supply of any other PGRs induce the inherent potential of the plant [31].

Still, researchers are working to find out the exact location of receptors for RPSA, these might be present in the cell wall, cell membrane or cytoplasm. Further it might be possible that RPSA could trigger the cascading effect, by inducing enzyme(s), or secondary messenger(s) (L (+)-adenosine) present on the cell membrane, on the plant metabolism and accumulation of intermediates of metabolic pathways that improved the overall performance of plant in regard to growth and production of secondary metabolites. In the present study, an enhancement in photosynthetic pigments was observed. The nitrogen content increased in the leaves of RPSA treated plants. It is well-established fact that an enhancement in N content favors the production of amino acids, proteins, and lipids that are the building blocks of chlorophylls and carotenoid content [31]. Almost similar findings were noted by Mollah *et al*. [28], in case of red amaranth leaves. In this study, data regarding leaf analysis indicated that RPSA supports the mineral nutrients absorption and utilization by increasing membrane permeability [32]. An increment in nutrient absorption plays a structural role in the production of biomass as indicated in growth parameters of treated plants. In the present study, CA activity significantly affected by RPSA treatment indicating RPSA has a definite role in the activation of Rubisco and PEP carboxylase enzymes. Carbon fixation rate has a direct relation with the activities of carboxylase and oxygenase functions of Rubisco [30]. An increment in nitrogen uptake by RPSA enhances the concentration of nitrate that might be responsible for increased NR activity. Similar positive effect of RPSA on activities of CA and NR was reported by Aftab *et al*. [9] and Naeem *et al*. [18].

Augmentation in the yield attributes by the application of RPSA credited to the role of RPSA in plant growth in general [28]. The promotive effect of RPSA on content and yield of essential oil might be due to its bioregulator effects on various plant processes including ion uptake, cell elongation, cell division, cell differentiation, sink/source regulation, enzymatic activities, protein synthesis and photosynthetic activity [33]. Moreover, the significant increase in the above-mentioned parameters of the RPSA treated plants might possibly culminate in the maximization of the herbage yield of the mint plant in the present study. Further, the improved herbage yield and dry matter production might have resulted due to enhanced water and nutrient uptake from the soil, followed by smooth translocation of photosynthates and other metabolites to the sinks in RPSA-treated plants employed.

Conclusions

The optimum level of RPSA was 100 mg L^{-1} for physiological activities, the content of essential oil of *M. arvensis* in the present study. The overall production of essential oil of the crop may be boosted by this emerging technique in an economical way as very little quantity of the radiated material is needed. The results obtained in pot experiment [29] were comparable to those conducted in the field, confirming that the effect of RPSA application proved true under both conditions. Additionally, the effect of foliar application of RPSA on *M. arvensis* in regard to growth, yield and essential oil content has been studied for the first time in field conditions. However, the phenomenon which stimulates the processes related to promotion of plant growth still needs further investigations.

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