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Naphthalene acetic acid and 6- benzylaminopurine induced phenolics, flavonoids and terpenoids in shoot cultures of *Mesosphaerum suaveolens* (L.) Kuntze and callus cultures of *Ocimum basilicum* L.

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Abstract

Mesosphaerum suaveolens (L.) Kuntze and *Ocimum basilicum* L. are sources of biologically important components like phenolics, flavonoids and terpenoids. These commonly growing wild plants with enormous medicinal value, have been mostly overlooked and used only as hedges for other economical crops. *In vitro* propagation of these plants would help to study the effect of different media components on important metabolites, including essential oils, pigments, antioxidants, food and flavouring agents which are of economic value. This study was focused on understanding the best suitable protocol and medium for the establishment of *in vitro* cultures of *Mesosphaerum suaveolens* and *Ocimum basilicum*. The work also focused on germination studies in both plants as a pre-requisite for culturing. The next focus was to understand how the culture conditions affected the amount of phenolics, flavonoids and terpenoids. Seedlings of *M. suaveolens* and fresh leaves of *O. basilicum* were inoculated and shoot cultures and callus cultures were obtained from them respectively, with different concentrations and combinations of Naphthalene Acetic Acid (NAA) and 6-Benzylaminopurine (BAP). The treatments used in the study for both plants were 0.5 mg/mL, 1.0 mg/mL, 1.5 mg/mL, 2.0 mg/mL, 2.5 mg/mL, 3.0 mg/mL, 3.5 mg/mL and 4.0 mg/mL each of NAA and BAP in combination. Among all the above combinations of the growth hormones on MS medium, 2.0 mg/mL combined concentration of NAA and BAP showed the highest biomass accumulation with the Total Phenolics Content (TPC) value of 17.58 ± 0.32 mg GAE/ g DW, Total Flavonoids Content (TFC) of 11516 ± 176.1 mg Quercetin/g DW and Total Terpenoids Content (TTC) of 80.25 ± 1.183 mg Linalool/g DW in *M. suaveolens* and 2.5 mg/mL combined concentration of NAA and BAP showed the highest biomass accumulation with the TPC value of 15.79 ± 0.13 mg GAE/g DW, TFC- 9513 ± 68.41 mg Quercetin/g DW and TTC of 75 ± 1.093 mg Linalool/g DW in *O. basilicum.* The levels of phenolics and terpenoids were higher in *M. suaveolens* whereas the levels of flavonoids were higher in *O. basilicum.* This study would help in further understanding other aspects of plant defense in these plants grown *in vitro.*

Keywords

Ocimum ; *Mesosphaerum* ; Secondary Metabolites; TPC; TFC; TTC

Introduction

Lamiaceae Martinov, the mint family, is known to be composed of more than 232 genera (1). The family is known for their unique aromatic characteristics due to the presence of essential volatile oils and their medicinal properties (2). The family includes various culinary plants such as *Salvia, Thymus, Mentha, Scutellaria, Melissa, Satureja, Lamium, Ocimum, Lavandula, Origanum, Mesosphaerum* and many more (3). *Mesosphaerum suaveolens* commonly, known as bush mint and locally known as vilayati tulsi, is a very understudied and underexplored plant that belongs to Lamiaceae family (4). *M. suaveolens* is known to contain many economically and biologically important secondary metabolites such as terpenes and terpenoids (monoterpenes, sesquiterpenes, diterpenes and triterpenes), phenolics, flavonoids, alkaloids, tannins, carotenoids, glycosides, steroids, quinones and many more (5). *M. suaveolens* is among the plants that have applications in various industries, such as food, pharmaceutical products, cosmetics and perfumery (6). *In vitro* culturing of *M. suaveolens* is done from various explants such as leaves, stems, roots etc. (7). Studies have shown that various plants from Lamiaceae, including *M. suaveolens* have been used for *in vitro* culture studies from seeds (8). The seeds form mucilage when soaked in water, which hampers their germination (9). *M. suaveolens* is a rigidly growing annual herb whose vegetative phase starts at the onset of the monsoon season and covers the nearby area due to reproducing successfully (10). The seeds of *M. suaveolens* have a huge demand in Mexico due to their medicinal and nutritive properties (11).

Ocimum basilicum L., also known as damro and sweet basil, is a very well-known plant of the Lamiaceae family. The essential oils from this plant have various applications in perfumery, cosmetics and many other medicinal uses (12). This plant has various important secondary metabolites such as terpenes, including monoterpenes, diterpenes, sesquiterpenes, phenylpropanoids, aldehydes, alcohols, fatty acids, phenolic compounds, antioxidant compounds such as caffeic acid, vanillic acid, rosmarinic acid, quercetin, rutin, apigenin and numerous essential oils (13). The extract of this plant has been used for treating skin infections, snake bites, acne, skin marks, insect stings, poor digestion, gastric illnesses, abdominal cramps, nausea and depression. (14). *O. basilicum* is an annual herb that flowers between July to October (monsoon onset) and continues to flower till the plant starts drying (15). This plant has a huge international market in Ethiopia because it has been used highly as a dried herb and spice (16).

Plant tissue culture is the technique of growing plant cells, tissues or organs under aseptic conditions. With the help of plant tissue culture techniques, secondary metabolites can be enhanced in the plants by treating the plants with different and multiple combinations of chemicals (17).

This study was undertaken to design the best suitable medium among MS and LS medium for the growth of *M. suaveolens* and *O. basilicum* in *in vitro* conditions by supplementing the medium with a combination of growth hormones (NAA and BAP). In this study, initially the seed germination studies were done for *M. suaveolens* and *O. basilicum*, but the seeds of *O. basilicum* did not turn into shoots and the germination rate was very poor. So, for *O. basilicum*, callus cultures were done. The study also focused on checking the levels of phenolics, flavonoids and terpenoids in the cultures and studying the relation between the growth regulators and the levels of secondary metabolites in both the plants. The findings of this study could be applied to understand other aspects of plant defence.

Materials and Methods

Field Studies

Collection and Identification of germplasm

Mesosphaerum suaveolens (L.) Kuntze and *Ocimum basilicum* L. were identified based on the morphological characters, followed by confirming with the herbarium sheet. The strong aroma of the plants added an advantage to the identification process. Both the plants are annual plants and grow throughout the year. *Ocimum basilicum* and *Mesosphaerum suaveolens* both have bluish-purple inflorescence and were collected from the roadside hedges of Bhayli, Vadodara, Gujarat, India.

Plant Authentication

Plant material was first collected, shade dried and pressed in between the newspapers to avoid moisture in the plant for about 7 days. The plant material was then subjected to poisoning by dipping the whole plant in a saturated solution of 0.1 % mercuric chloride (HiMedia, #GRM1067, India) in 70 % ethyl alcohol (HiMedia, #MB106, India) (18). Then, after drying the plant was mounted on the herbarium sheet and on the bottom right corner of the sheet, details of the plant such as the scientific name, common name, family name, place and season of collection were added. The herbarium sheet was then submitted to and authenticated by BARO Herbarium, The Maharaja Sayajirao University of Baroda, Gujarat.

Studies on Seed Germination

Germination using different soils

The seeds were sown in different soils taken from different areas around the college campus. In the first set, 50 g of soil was removed and used from the original *M. suaveolens* plant location, 5 cm below the surface. The second set contained fertile garden soil from the university garden, 5 cm below the surface. The third soil set was taken from campus soil, from the edges of the university basketball court, 5 cm below the surface and the last set contained coco peat commercially purchased from the nursery. All the sets were sprinkled with little water daily to keep the sets moistened. All the sets were kept for 7-10 days to allow the seeds to sprout.

Germination on Basal Media

The seeds were first washed with distilled water. Then, the seeds were surface sterilized with 0.01 % $HgCl_2$ (HiMedia,

#GRM1067, India) for 2 min. Then, 2 min wash of 70 % ethanol (HiMedia, #MB106, India) was given. Then, the seeds were thoroughly washed with autoclaved distilled water thrice. Then, the seeds were placed onto the basal medium with the help of forceps. The Basal Medium used here was Murashige and Skoog (MS) Medium (HiMedia, #PT018, India) with 3 % sucrose and 0.8 % Phytoagar. The pH of the medium was set at 5.7. Thereafter, the seeds were allowed to germinate for about 30 days. The cultured flasks were kept in the plant growth room at 24 ˚C ± 2 ˚C. The seeds were continuously and daily observed to learn the germination pattern (19).

Overnight treatment on filter paper

The filter paper was moistened by spraying with water and kept on the petri plate. The seeds were then washed and placed on the filter paper. The lid of the petri plate was closed and then kept overnight for germination (in dark). The seed set was kept for around 3 days for germination in dark (20).

Mechanical scarification of the seeds

The seeds were first scratched with the help of forceps then the seeds were scarified by means of sandpaper. Once the outer coating was partially removed, the seeds were then placed on filter paper in a petri plate. Thereafter, the filter paper was moistened with water and then incubated overnight in dark. The plate was kept for 3 days for germination. On the second day, the mucus of the seeds was removed and kept on the filter paper for germination (21).

Establishment of in vitro cultures of Ocimum basilicum and Mesosphaerum suaveolens on MS and LS Media

Preparation of the Growth Media

The plants were set to acclimatize on 2 basal medium, Murashige and Skoog (MS) medium (HiMedia, #PT018, India) and Linsmaier and Skoog (LS) medium (HiMedia, #PT040, India). The medium was supplemented with 1.5 % Sucrose and the pH of the medium was set at 5.7. Phytoagar concentration used for the solid cultures was 0.8 %. Auxin and cytokinin used as growth hormones were Naphthalene acetic acid (NAA) and 6-Benzylaminopurine (BAP) respectively. The concentrations of the growth hormones used for acclimatization of the plants were Control, 0.5 mg/mL, 1 mg/mL, 1.5 mg/mL, 2 mg/mL, 2.5 mg/mL, 3.5 mg/mL and 4 mg/mL each for NAA and BAP in combination. The medium was then autoclaved for 15 min at 121 ˚C temperature and 15 psi pressure (22). The sets were run in triplicates.

Sterilization and Inoculation

The 7 days old seedlings of *M. suaveolens* were taken for sterilization process and inoculation into the basal MS Medium (HiMedia, #PT018, India). The seedlings were first taken in an autoclaved flask and washed with 0.01 % $HgCl_2$ (HiMedia, #GRM1067, India) for 2 min followed by washing with 70 % ethanol (HiMedia, #MB106, India) for 2 min. Then, 3 washes of autoclaved distilled water were given (2 min each) to remove all the traces of the applied chemicals. The seedlings were then placed onto basal MS medium (HiMedia, #PT018, India) and LS medium (HiMedia, #PT040, India) prepared with different concentrations of the growth hormones (23). *O. basilicum* seeds did not germinate into seedlings, hence leaf samples were collected from the roadside hedges of Bhayli, Vadodara. Fresh leaves of *O. basilicum* were washed with 0.01 % $HgCl_2$ (HiMedia, #GRM1067, India) in a flask for 2 min. Thereafter, the leaves were washed with 70 % ethanol (HiMedia, #MB106, India) for 2 min. The leaf explants were cut and then placed onto the basal MS medium (HiMedia, #PT018, India) and LS Medium (HiMedia, #PT040, India) prepared with different concentrations of the growth hormones (24). The *in vitro* cultures were maintained in the plant culture room where the temperature of the room was set at 24 ˚C ± 2 ˚C. The photoperiod was set for 16 h light and 8 h dark provided with the help of white, fluorescent tubes whose intensity were set at 1000 lux. The cultures were kept and observed for 4 weeks after which they were used for subculturing. After 4 weeks the cultures from individual concentrations were subjected for the testing of Total Phenolic Content (TPC), Total Flavonoid Content (TFC) and Total Terpenoid Content (TTC).

Levels of secondary metabolites in in vitro cultures of Ocimum basilicum and Mesosphaerum suaveolens

Preparation of the Plant Extract from the cultures

The *in vitro* raised seedling materials (shoot cultures) and the leaf raised calluses, all were taken and air dried at room temperature and then subjected to mortar and pestle. 0.5 g of the powder from each plant extract were then taken and refluxed with methanol in waterbath at 45 °C for 3 h. The extract from both the plants were then separately filtered through Whatman filter paper No. 4. The collected filtrates were then dried on Rotary evaporator and preserved for further analysis for the determination of phenolics, flavonoids and terpenoids. Few modifications were done in the protocol of El-Baz (25).

Total Phenolic Content

The Total Phenolics Content (TPC) was performed with the Folin-Ciocalteu reagent by the method (26) in which few modifications were done. 10 mg of the plant extract was dissolved in 10 mL of methanol (1 mg/mL) and then 0.5 mL FC Reagent (Folin-Ciocalteu reagent) was added to it (FC: Water- 1:1) in the reaction tube. The samples were then mixed properly and incubated at room temperature for 3 min. Thereafter, 2 mL of 20 % sodium carbonate was added to the reaction tubes and then the tubes were vortexed properly and kept in dark and incubated for 30 min. The absorbance of the samples was measured at 650 nm using microcontroller-based UV- Visible spectrophotometer CL-1320 Chemiline against a blank containing distilled water. The standard curve was plotted with the different concentrations of gallic acid in methanol. Total phenolic content was expressed as mg of Gallic acid equivalent (GAE) per g of dry weight (mg GAE/g DW).

$$C = cV/m$$

Where, C = Total Phenolic Content mg GAE/ g dry weight, c = concentration of gallic acid obtained from calibration curve in mg/mL, V = Volume of extract in mL, and m = mass of extract in g.

Total Flavonoid Content

The Total Flavonoids Content (TFC) was performed by the standard method (27). 0.1 mL of the plant extract was added to 0.3 mL distilled water. Then, 30 mL of 5 % $NaNO_2$ was added to the tube. Then, the sample was incubated for 5 min at the room temperature. Then, 0.2 mL of 1 mM NaOH was added to the tube and then 0.34 mL of distilled water was added to the tube and the absorbance was measured at 510 nm using Microcontroller based UV- Visible Spectrophotometer CL-1320 Chemiline. The standard curve was plotted with different concentrations of quercetin in ethanol (HiMedia, #MB106, India). Total flavonoids content was expressed as mg of quercetin equivalent per g of dry weight (mg Quercetin/g DW).

$$C = cV/m$$

Where, C = Total Flavonoid Content mg Quercetin/ g dry weight, c = concentration of quercetin obtained from calibration curve in mg/mL, V = Volume of extract in mL, and m = mass of extract in g

Total Terpenoid Content

The Total Terpenoid Content (TTC) was determined by the standard method (28). 1 mL of plant extract was taken and 2 mL of chloroform was added to it. The sample mixture was properly mixed and then kept at room temperature for 3 min. Then, 200 µL of concentrated sulfuric acid was added into the mixture and then incubated at room temperature in dark for 2 h. After this step, reddish-brown precipitate was found in the tube which was kept undisturbed. Then, the supernatant from the tube was carefully removed and then 3 mL of methanol was added to it. The tubes were shaken properly till the precipitate dissolved completely in methanol. The absorbance was measured at 538 nm using a Microcontroller based UV- Visible Spectrophotometer CL-1320 Chemiline. The standard curve was plotted with different concentrations of linalool in methanol. Total terpenoids content were determined as mg of linalool equivalent per g of dry weight (mg Linalool/ g DW) (29).

$$C = cV/m$$

Where, C = Total Terpenoid Content mg Linalool/ g dry weight, c = concentration of linalool obtained from calibration curve in mg/mL, V = Volume of extract in mL, and m = mass of extract in g.

Statistical Analysis

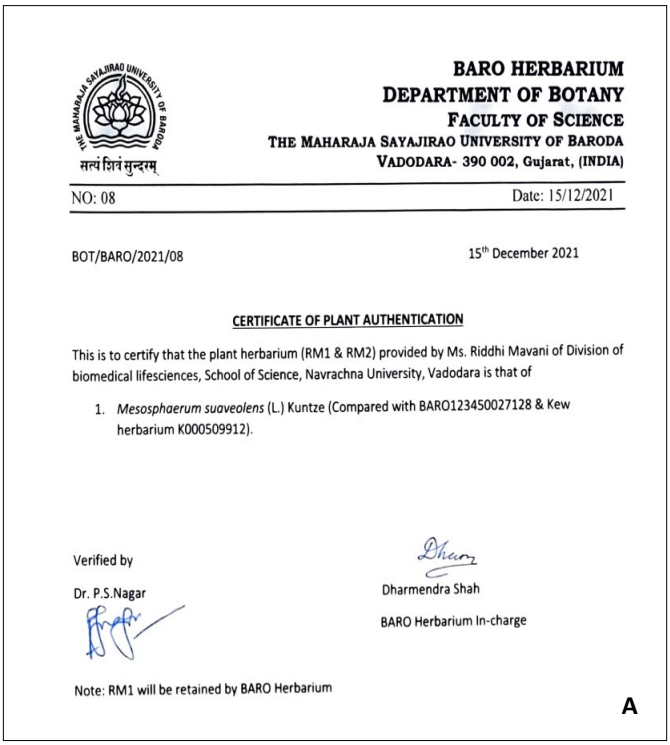
The statistical analysis for all the sets were done in triplicates using one way ANOVA, followed by Tukeys test comparison and significance was noted at *p<0.05 and **p<0.01. The software used for the analysis was GraphPad Prism 8.4.2.

Results and Discussion

Field Studies

Plant Authentication

The authentication of *M. suaveolens* and *O. basilicum* was done at BARO Herbarium, The Maharaja Sayajirao University of Baroda, Vadodara, Gujarat, India. The voucher specimens of *M. suaveolens* and *O. basilicum* (RM1 & RM2) and (RM3 & RM4) respectively were submitted for authentication. The specimens *M. suaveolens* and *O. basilicum* were examined with BARO123450027128, K000509912! and BARO123450026912, K000479656! respectively (Fig. 1).



BARO HERBARIUM
DEPARTMENT OF BOTANY
FACULTY OF SCIENCE
THE MAHARAJA SAYAJIRAO UNIVERSITY OF BARODA
VADODARA- 390 002, Gujarat, (INDIA)

NO: 08 Date: 15/12/2021

BOT/BARO/2021/08 15th December 2021

CERTIFICATE OF PLANT AUTHENTICATION

This is to certify that the plant herbarium (RM1 & RM2) provided by Ms. Riddhi Mavani of Division of biomedical lifesciences, School of Science, Navrachna University, Vadodara is that of

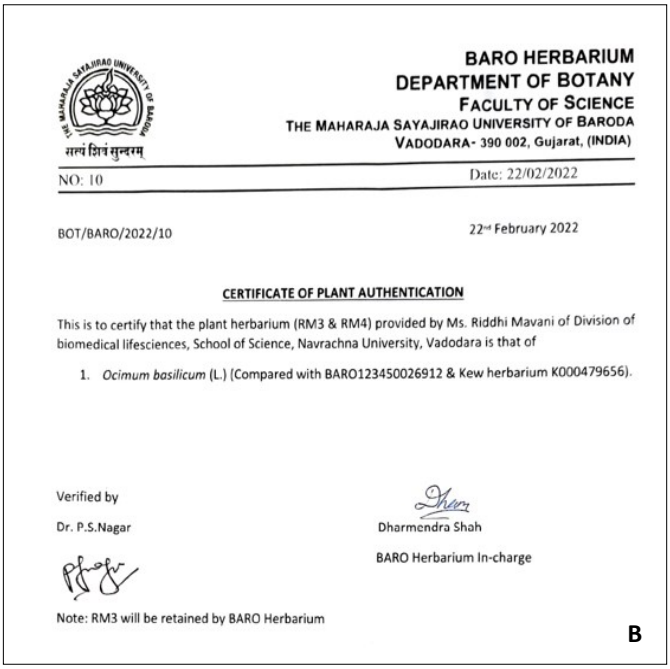
1. *Mesosphaerum suaveolens* (L.) Kuntze (Compared with BARO123450027128 & Kew herbarium K000509912).

Verified by
Dr. P.S.Nagar

Dharmendra Shah
BARO Herbarium In-charge

Note: RM1 will be retained by BARO Herbarium

A



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NO: 10 Date: 22/02/2022

BOT/BARO/2022/10 22nd February 2022

CERTIFICATE OF PLANT AUTHENTICATION

This is to certify that the plant herbarium (RM3 & RM4) provided by Ms. Riddhi Mavani of Division of biomedical lifesciences, School of Science, Navrachna University, Vadodara is that of

1. *Ocimum basilicum* (L.) (Compared with BARO123450026912 & Kew herbarium K000479656).

Verified by
Dr. P.S.Nagar

Dharmendra Shah
BARO Herbarium In-charge

Note: RM3 will be retained by BARO Herbarium

B

Fig. 1. A. Authentication certificate of *Mesosphaerum suaveolens* and **B**. Authentication certificate of *Ocimum basilicum*.

Seed Germination studies by different methods

Seed Germination

Different methods were used for the germination of the seeds. The germination was first tried by sowing the seeds on soil samples from different locations. The seeds were kept for around 30 days, but no visible germination was seen in any of the soil samples. The seeds had formed mucilage in the water, which is one of the important proper-

ties of the seeds of *M. suaveolens*. Next, the germination was tried by incubating the seeds on the filter paper overnight. Here, the same problem existed, the seeds were not able to germinate within 3 days and the mucilage was seen around the seeds when the filter paper was moistened.

Another method was tried for seed germination, in which after the sterilization of the seeds, they were inoculated onto the MS medium. In this method as well, the seeds formed mucilage. The seeds were not able to germinate even after 40 days.

Mechanical scarification is the method in which the seeds are physically scraped with the help of sandpaper to increase the water imbibition of the seeds (30). The seeds germinated within 24 h after placing the scarified seeds on to the filter paper. Three days were required for all the seeds to completely sprout. The major hindrance was found to be the mucilage formation which when removed, allowed the seeds to germinate (Fig. 2). The seed germination was 72 %.

Establishment of in vitro cultures of Ocimum basilicum L. and Mesosphaerum suaveolens (L.) Kuntze on LS and MS Media

The cultures were subjected to the same growth parameters and then the growth pattern was compared between the 2 different basal media MS and LS medium. Table 1 shows the number of seedlings inoculated on MS and LS medium and the number of shoots formed from them in *M. suaveolens* and the number of leaves inoculated to MS and LS medium and number of calluses formed in *O. basilicum.* It was found that MS medium was more suitable than LS medium when growth parameters were taken into consid-

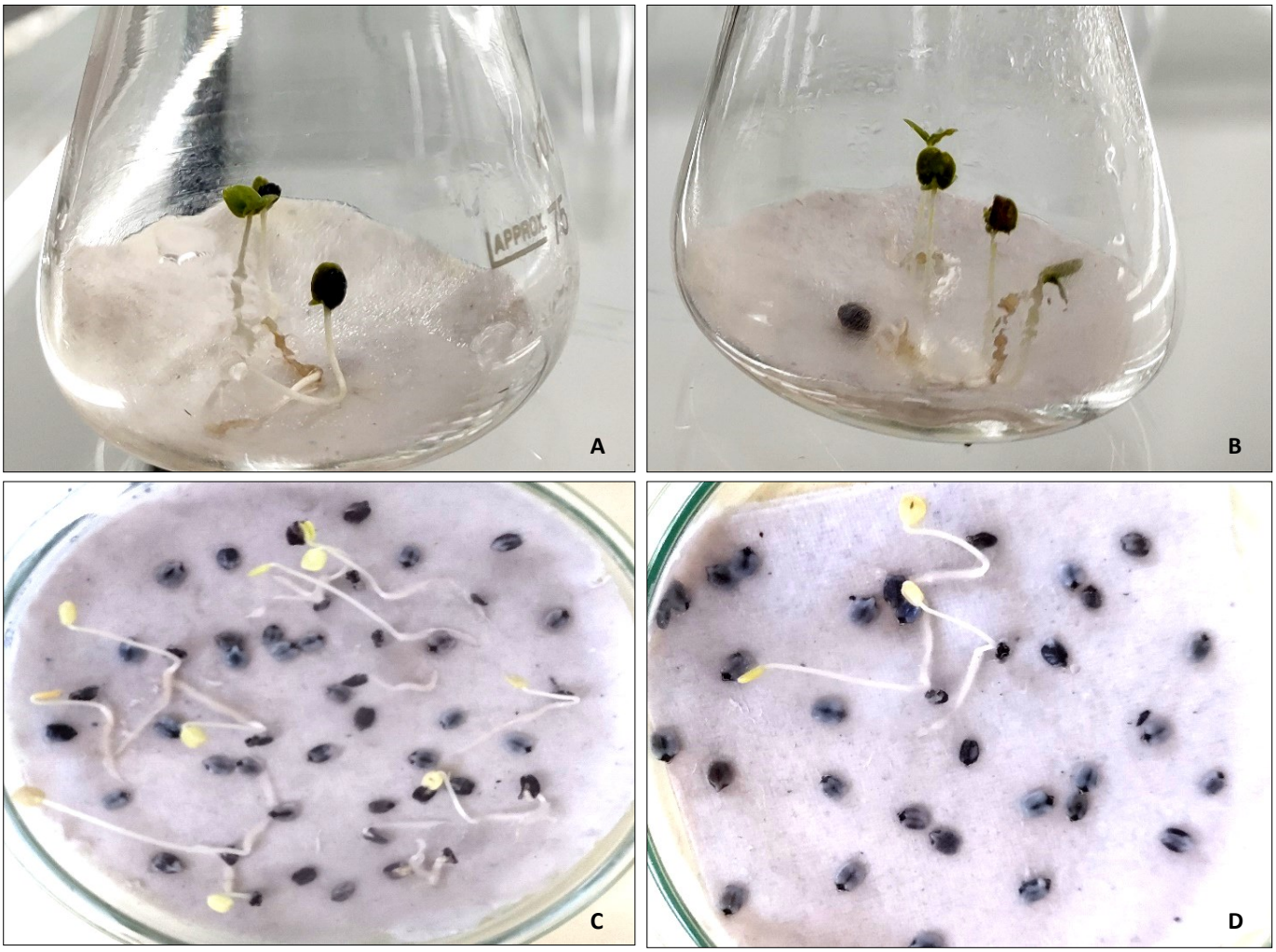

Fig. 2. A, B,& D. Different sets of seed germination of *Mesosphaerum suaveolens* through mechanical scarification, **C.** showed the highest % of germination (72 %).

Table 1. The table depicts the tabular format for the experimental setup of number of seedlings and leaves inoculated and the numbe r of shoots and calluses formed from them respectively in *M. suaveolens* and *O. basilicum.*

Sl. No.	Plant Name	No. of explants inoculated	No. of explants which turned into callus	No. of seedlings inoculated	No. of seedlings which turned into shoots
1.	*Mesosphaerum suaveolens* (L.) Kuntze. On MS Medium	NA	NA	98 (2 sets)	82
2.	*Ocimum basilicum* L. on MS Medium	98 (2 sets)	84	NA	NA
3.	*Mesosphaerum suaveolens* (L.) Kuntze. On LS Medium	NA	NA	98 (2 sets)	76
4.	*Ocimum basilicum* L. on LS Medium	98 (2 sets)	75	NA	NA

2 sets of 49 tubes were kept in triplicates of each concentration.

eration. In one of the similar studies in the species of Lamiaceae family, *Satureja thymbra* L., the best initiation of culture was seen in MS medium when compared to LS medium (31). Both the callus and the shoot growth in this study was better in MS Medium. The callus grown were fleshier and more greenish when compared with that of the callus grown in LS Medium. The callus of *O. basilicum* grew more in width in MS Medium and the seedling of *M. suaveolens* also grew more in length in the MS Medium. It was found that the growth of *M. suaveolens* was best seen in the MS Medium when supplemented with growth hormones NAA and BAP at the concentration of 2.0 mg/mL (Fig. 3 and 4). The growth of *O. basilicum* was best seen in the MS Medium with the concentration of growth hormones NAA and BAP of 2.5 mg/mL (Fig. 5 and 6). The combined effects of 2 auxins have shown better growth in few plants of Lamiaceae when compared to the effect of a single auxin (31). Auxin and Cytokinin when combined in proper and different combination have shown to initiate shoot and root induction in *O. basilicum* (32). Slightly purplish coloured calluses were seen in few concentrations of *O. basilicum* which may be due to the secretion of anthocyanins in the epidermal layer of the leaf, which shows antioxidative effect as well (33). Callus culture growth is mainly dependent on the biomass and yield accumulation. At higher concentrations, the calluses are shown to have poor growth while at lower concentrations, the calluses seem to have good growth, the reason mostly being the growth hormone concentration and nutrients in the medium (34). In the shoot cultures of *M. suaveolens,* after few days, blackening of the cultures/shoots were seen (Fig. 3 and 4), that is because of the increase in the level of phenolics (35).

Levels of secondary metabolites in in vitro cultures of Ocimum basilicum L. and Mesosphaerum suaveolens (L.) Kuntze

Total Phenolic Content

The Total Phenolic Content (TPC) in *M. suaveolens* was done for all the samples grown with different concentrations of growth hormones in MS and LS medium, where the concentration of 2.0 mg/mL, 2.5 mg/mL and 3.0 mg/mL showed the highest phenolics among which the concentration of 2.0 mg/mL showed the highest phenolic content of 17.58 ± 0.32 mg GAE/ g DW of GAE and the concentrations 1.0 mg/mL, 1.5 mg/mL, 3.5 mg/mL and 4.0 mg/mL showed average phenolic content in MS medium. While the seedling of *M. suaveolens* grown in LS medium showed the highest phenolic content in 2.5 mg/mL concentration while the concentration of 2.0 mg/mL and 3.0 mg/mL showed average phenolic content. The maximum phenolic content was found to be 8.694 ± 0.35 mg GAE/ g DW of GAE at the concentration 2.5 mg/mL of BAP and NAA. In *Ocimum basilicum* L. both MS and LS medium showed highest amount of phenolic content at a concentration of 1.5 mg/mL, 2.0 mg/mL, 2.5 mg/mL and 3.0 mg/mL where the maximum phenolic content was found to be 15.79 ± 0.13 mg GAE/ g DW of GAE at concentration 2.5 mg/mL of NAA and BAP. On comparing the total phenolics in MS and LS medium, MS medium was found to be a better medium for the growth of plants as well as the amount of total phenolic content released (Fig. 7). In previous studies, total phenolic content in *in vitro* cultured leaves, stems and roots showed higher values when compared to the field grown leaves, stems and roots in both *O. basilicum* and *O. tenuiflorum* L. (Lamiaceae) (36). The levels of phenolics decrease at higher concentrations of growth hormones (37) and similar results were also seen in this study.

Total Flavonoid Content

The Total Flavonoid Content (TFC) in *M. suaveolens* was done for all the samples grown with different concentrations of growth hormones in MS and LS medium, where the concentration of 2.0 mg/mL and 3.0 mg/mL showed the highest flavonoid among which the concentration of 2.0 mg/mL showed the highest flavonoid content of 11516 ± 176.1 mg Quercetin/ g DW of Quercetin and the concentrations 0.5 mg/mL, 1.0 mg/mL, 1.5 mg/mL, 2.5 mg/mL, 3.5 mg/mL and 4.0 mg/mL showed average flavonoid content in MS medium. While the seedling of *M. suaveolens* grown in LS medium showed the highest flavonoid content in 2.5 mg/mL concentration while the concentration of 2.0 mg/mL and 3.0 mg/mL showed average flavonoid content. The maximum flavonoid content was found to be 5666 ± 102.5 mg Quercetin/ g DW of Quercetin at the concentration 2.5 mg/mL of BAP and NAA. *O. basilicum* in both MS and LS medium showed highest amount of flavonoid content in the concentration of 1.5 mg/mL, 2.0 mg/mL, 2.5 mg/mL, 3.0 mg/mL and 3.5 mg/mL where the maximum flavonoid content was found to be 9513 ± 68.41 mg Quercetin/ g DW of Quercetin in MS medium and 6870 ± 96.03 mg Quercetin/ g DW of Quercetin in LS medium at the concentration 2.5 mg/mL of NAA and BAP. On comparing the total flavonoid in MS and LS medium, MS medium was found to be a better medium for the growth of plants as well as the amount of total flavonoid content released (Fig. 8). Also, the levels of flavonoids were secreted in the highest amounts when compared to phenolics and terpenoids in both *M. suaveolens* and *O. basilicum*. After the addition of various growth regulators, a 91.54 % augmentation in the levels of flavonoids was seen in *Mentha* × *piperita* L. (Lamiaceae) (38). With the manipulation of various growth regulators and auxins, increased levels of flavonoids were also seen in *Salvia moorcroftiana* Wall. ex Benth. (Lamiaceae) and an increase in the levels of flavonoids leads to high antioxidant, antimicrobial and anti-inflammatory activities (39). Similar results were also found in the plant *Scutellaria brevibracteata* Stapf (Lamiaceae) where diverse flavones were secreted in *in vitro* cultures (40). Flavonoids have a symphony only with the auxins and the auxins then regulate the cytokinin actions and both of them together are responsible for root and shoot formation (41).

Total Terpenoid Content

The Total Terpenoid Content (TTC) in *M. suaveolens* was done for all the samples grown with different concentrations of growth hormones in MS and LS medium, where the concentration of 2.0 mg/mL showed the maximum terpenoid content with 80.25 ± 1.183 mg Linalool/g DW of

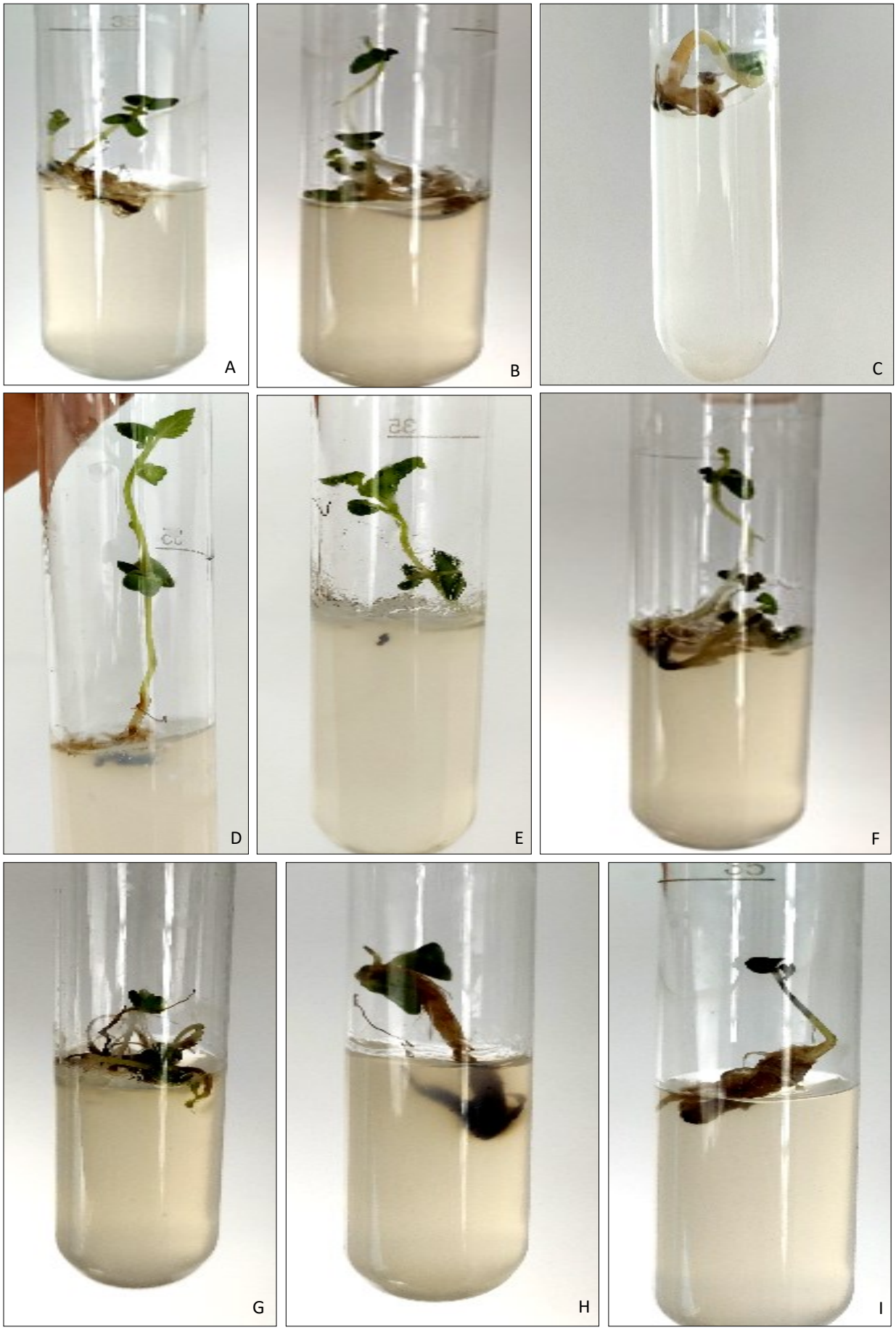

Fig. 3. The seedlings of *Mesosphaerum suaveolens* grown on MS Medium supplemented with different concentration of growth hormones in equal and combined combination (NAA and BAP): **A**. Control, **B**. 0.5 mg/mL, **C**. 1.0 mg/mL, **D**. 1.5 mg/mL, **E**. 2.0 mg/mL, **F**. 2.5 mg/mL, **G**. 3.0 mg/mL, **H**. 3.5 mg/mL and **I**. 4.0 mg/mL.

Linalool in MS and 67.18 ± 0.88 mg Linalool/g DW of

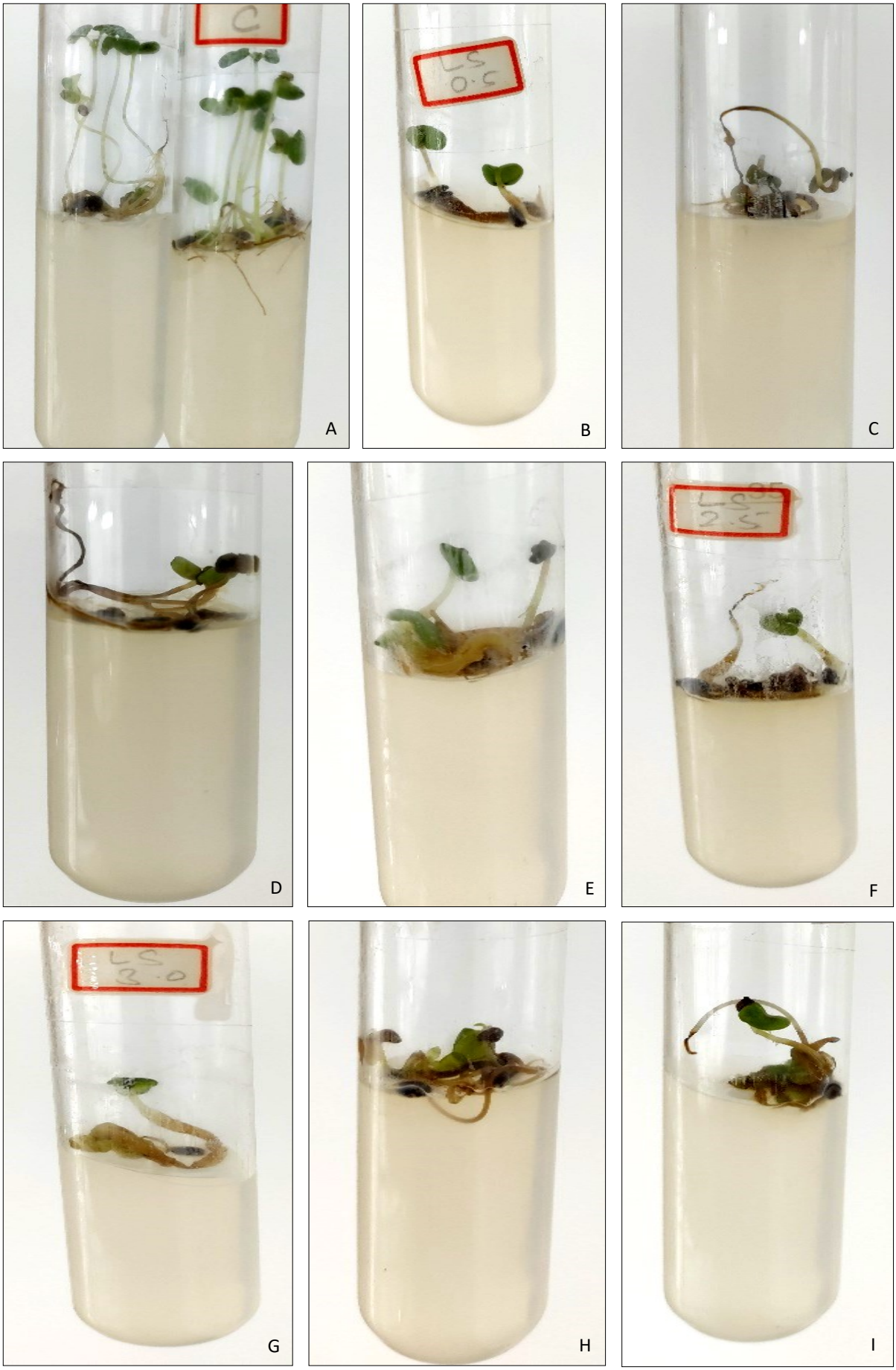

Fig. 4. The seedlings of *Mesosphaerum suaveolens* grown on LS Medium supplemented with different concentration of growth hormones in equal and combined combination (NAA and BAP): **A**. Control, **B**. 0.5 mg/mL, **C**. 1.0 mg/mL, **D**. 1.5 mg/mL, **E**. 2.0 mg/mL, **F**. 2.5 mg/mL, **G**. 3.0 mg/mL, **H**. 3.5 mg/mL and **I**. 4.0 mg/mL.

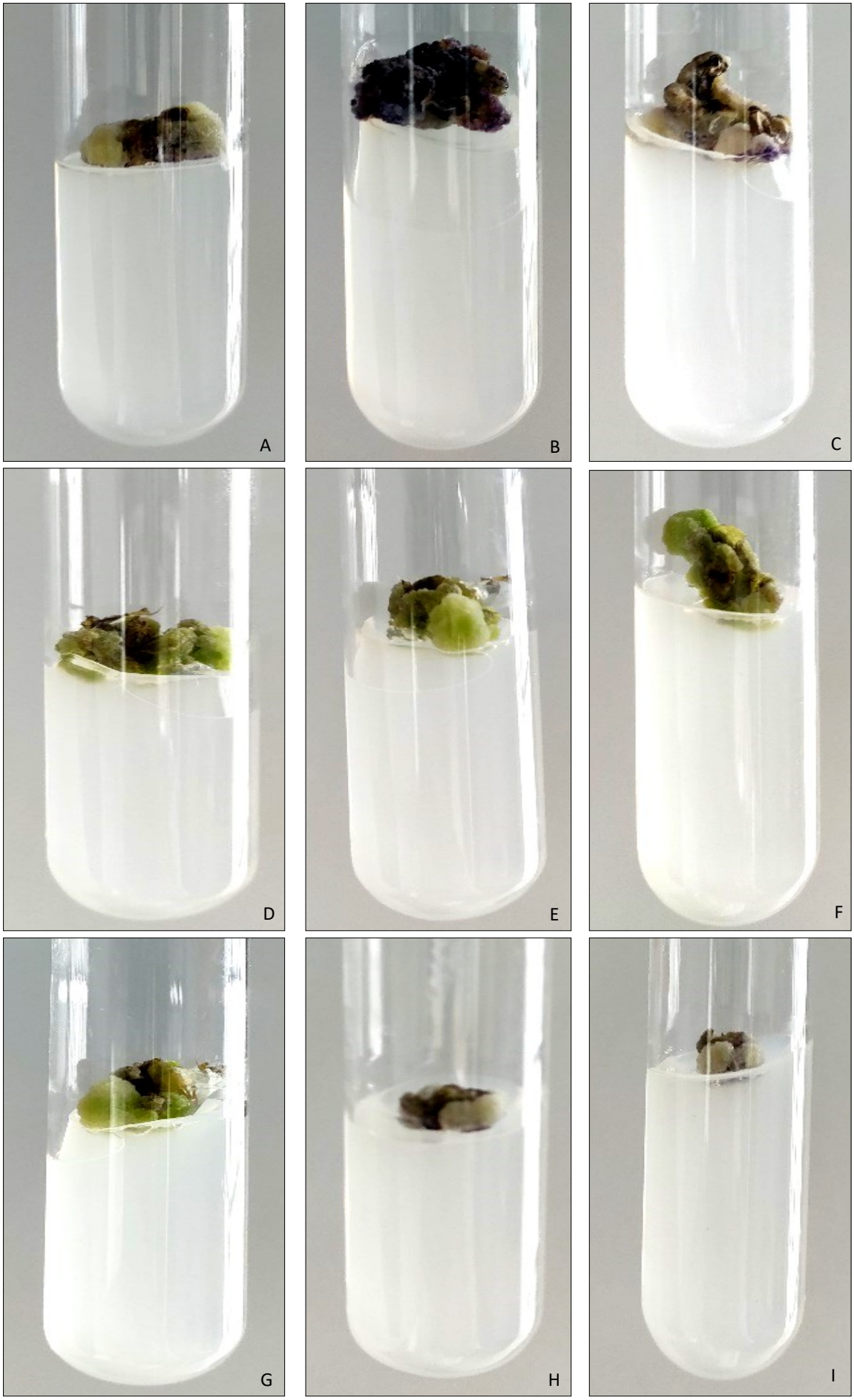

Fig. 5. (Starting from left to right) The callus of *Ocimum basilicum* grown on MS Medium supplemented with different concentration of growth hormones in equal and combined combination (NAA and BAP): **A**. Control, **B**. 0.5 mg/mL, **C**. 1.0 mg/mL, D. 1.5 mg/mL, **E**. 2.0 mg/mL, **F**. 2.5 mg/mL, **G**. 3.0 mg/mL, **H**. 3.5 mg/mL and **I**. 4.0 mg/mL.

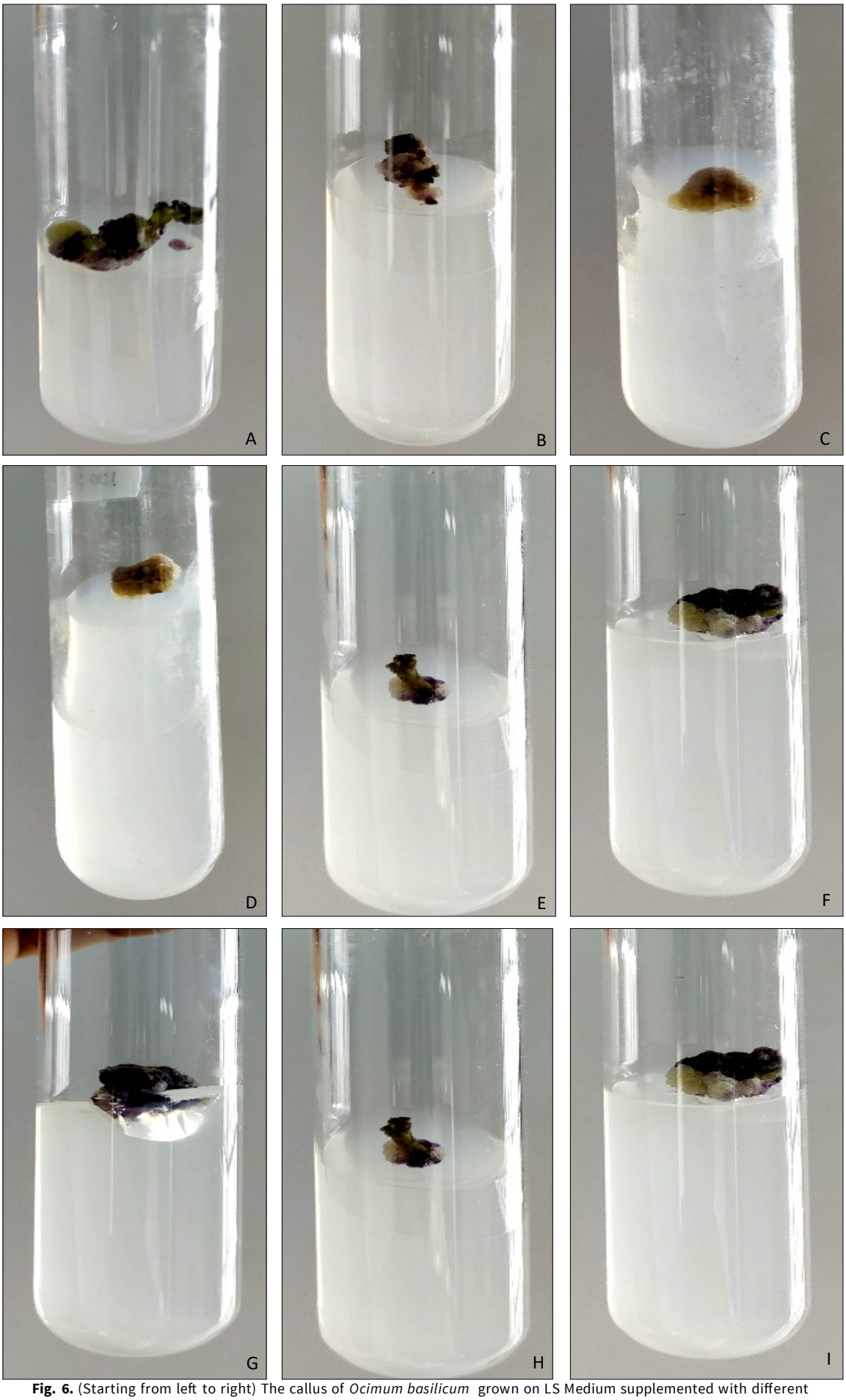

Fig. 6. (Starting from left to right) The callus of *Ocimum basilicum* grown on LS Medium supplemented with different concentration of growth hormones in equal and combined combination (NAA and BAP): **A**. Control, **B**. 0.5 mg/mL, **C**. 1.0 mg/mL, **D**. 1.5 mg/mL, **E**. 2.0 mg/mL, **F**. 2.5 mg/mL, **G**. 3.0 mg/mL, **H**. 3.5 mg/mL and **I**. 4.0 mg/mL.

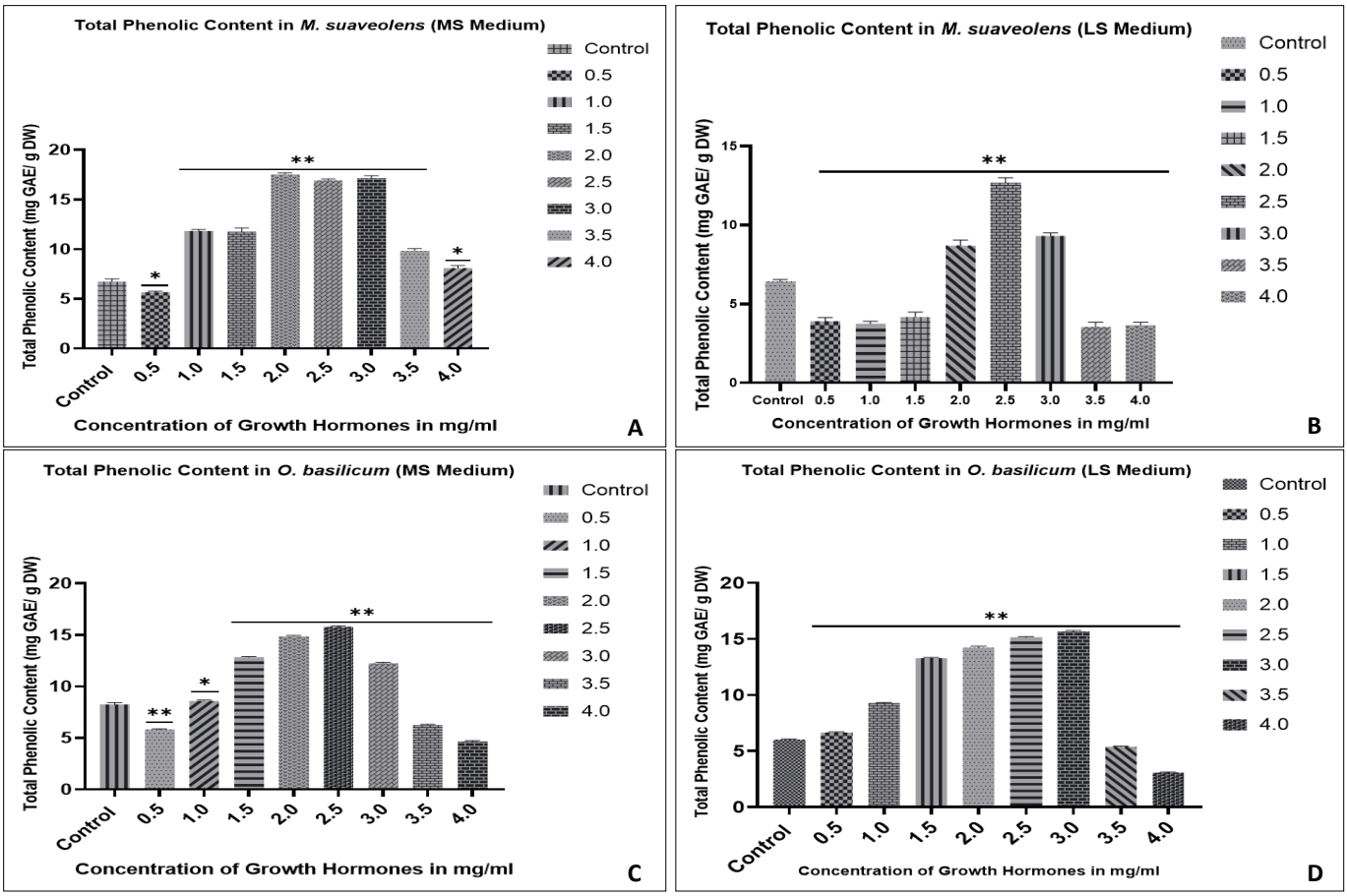

Fig. 7. Total Phenolic Content in **A**. *Mesosphaerum suaveolens* grown on MS Medium **B**. *Mesosphaerum suaveolens* grown on LS Medium **C**. *Ocimum basilicum* grown on MS Medium and **D**. *Ocimum basilicum* grown on LS Medium where *p<0.05 and **p<0.01.

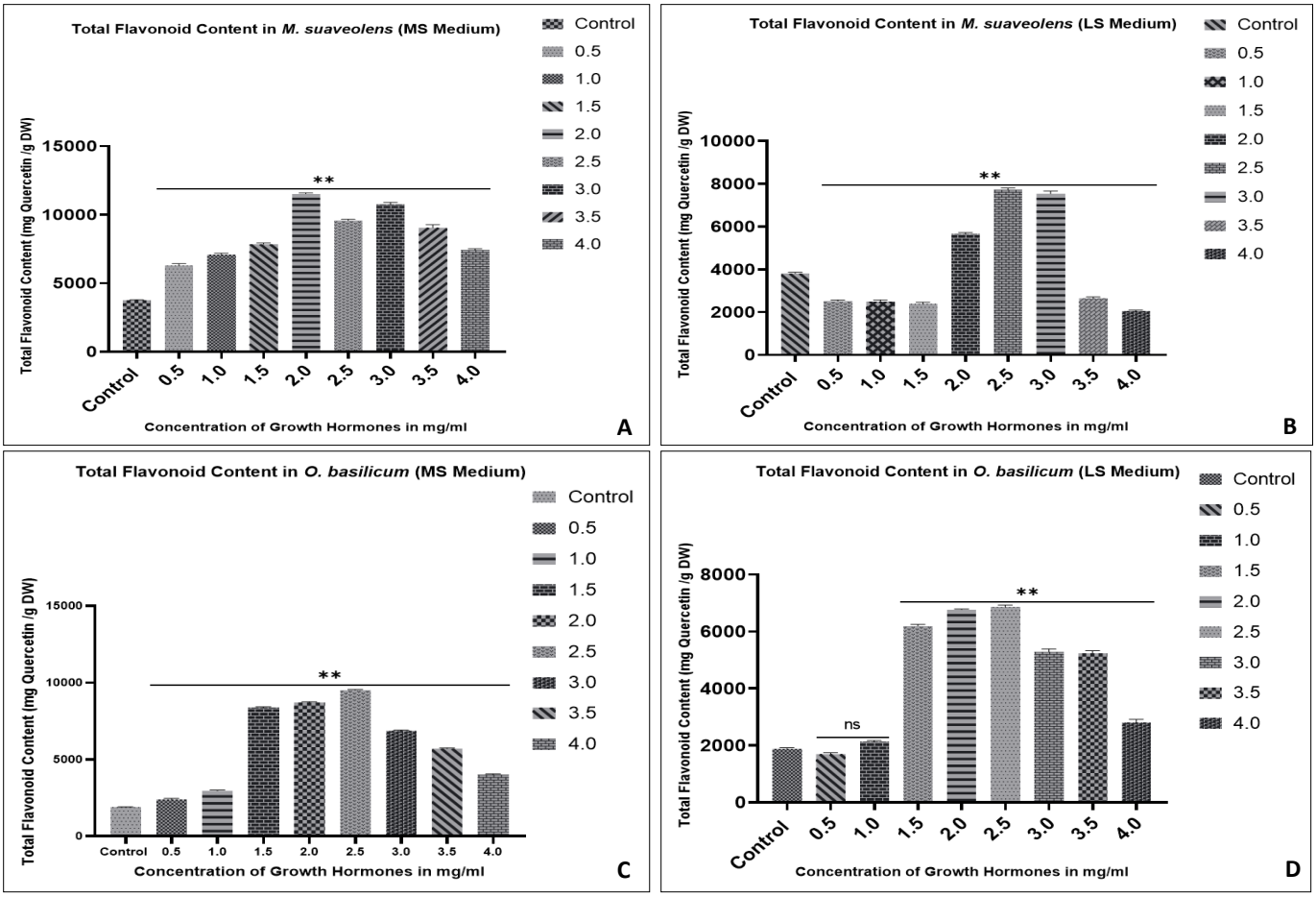

Fig. 8. Total Flavonoid Content in **A**. *Mesosphaerum suaveolens* grown on MS Medium **B**. *Mesosphaerum suaveolens* grown on LS Medium **C**. *Ocimum basilicum* grown on MS Medium and **D**. *Ocimum basilicum* grown on LS Medium where ns represents non-significant value, *p<0.05 and **p<0.01.

Linalool in LS medium at the concentration of 2.5 mg/mL of NAA and BAP. *O. basilicum* in both MS and LS medium showed the highest amount of terpenoid content in the concentration of 2.0 mg/mL and 2.5 mg/mL, where the maximum terpenoid content was found to be 80.28 ± 1.154 mg Linalool/g DW of Linalool in MS Medium at the concentration of 2.5 mg/mL of NAA and BAP and 70.61 ± 1.093 mg Linalool/g DW of Linalool in LS Medium at the concentration of 2.5 mg/mL of NAA and BAP. On comparing the total terpenoid content in MS and LS medium, MS medium was found to be a better medium for the growth of plants as well as the amount of total terpenoid content released (Fig. 9). The terpenoids were secreted in moderate amounts when compared to flavonoids, but still higher than the phenolics in both plants. High terpenoid and essential oil components have been shown to induce antimicrobial activity, insecticidal activity, repellent activity and larvicidal activity in *Mesosphaerum suaveolens* (42). High terpenoid and essential oil components have also been shown to have antioxidant, antimicrobial and mosquito larvicidal activity in *O. basilicum* and *O. americanum* L. (43). The addition of plant growth regulators such as NAA and BA to the medium has shown significant results in callus development, callus multiplication and cell differentiation. It also shows higher antioxidant properties which are mainly due to the production of secondary metabolites such as phenolic compounds and terpenes in *Ziziphora tenuior* L. (Lamiaceae) (44). The isoprenoid pathway of cytokinin and the terpenoid biosynthesis pathways are known to overlap when the medium is supplemented with only cytokinin, but when auxin is added in combination with cytokinin to the medium, more monoterpenes and sesquiterpenes are formed, thereby increasing the levels of terpenoids (45). Similar results were seen in *Coleus comosus* Hochst. ex Gürke (Lamiaceae), where increased levels of terpenes such as isoprenes, monoterpenes and diterpenes were found when the medium was supplemented with both NAA and BAP (46).

Conclusion

The germination studies in *M. suaveolens* as well as *O. basilicum* revealed that mechanical scarification was the only method to obtain a good % of germination. The *in vitro* culture studies of both plants showed that MS medium was found to be a better growth medium than LS medium. The best suited hormone combination for both plants was NAA and BAP. Both *M. suaveolens* as well as *O. basilicum* have various medicinal uses, so in order to exploit their proper use in health supplements in the best quality and quantity, it is necessary to get their yield in maximum amounts by altering the combination of plant growth hormones. It was also seen that in concentrations where there

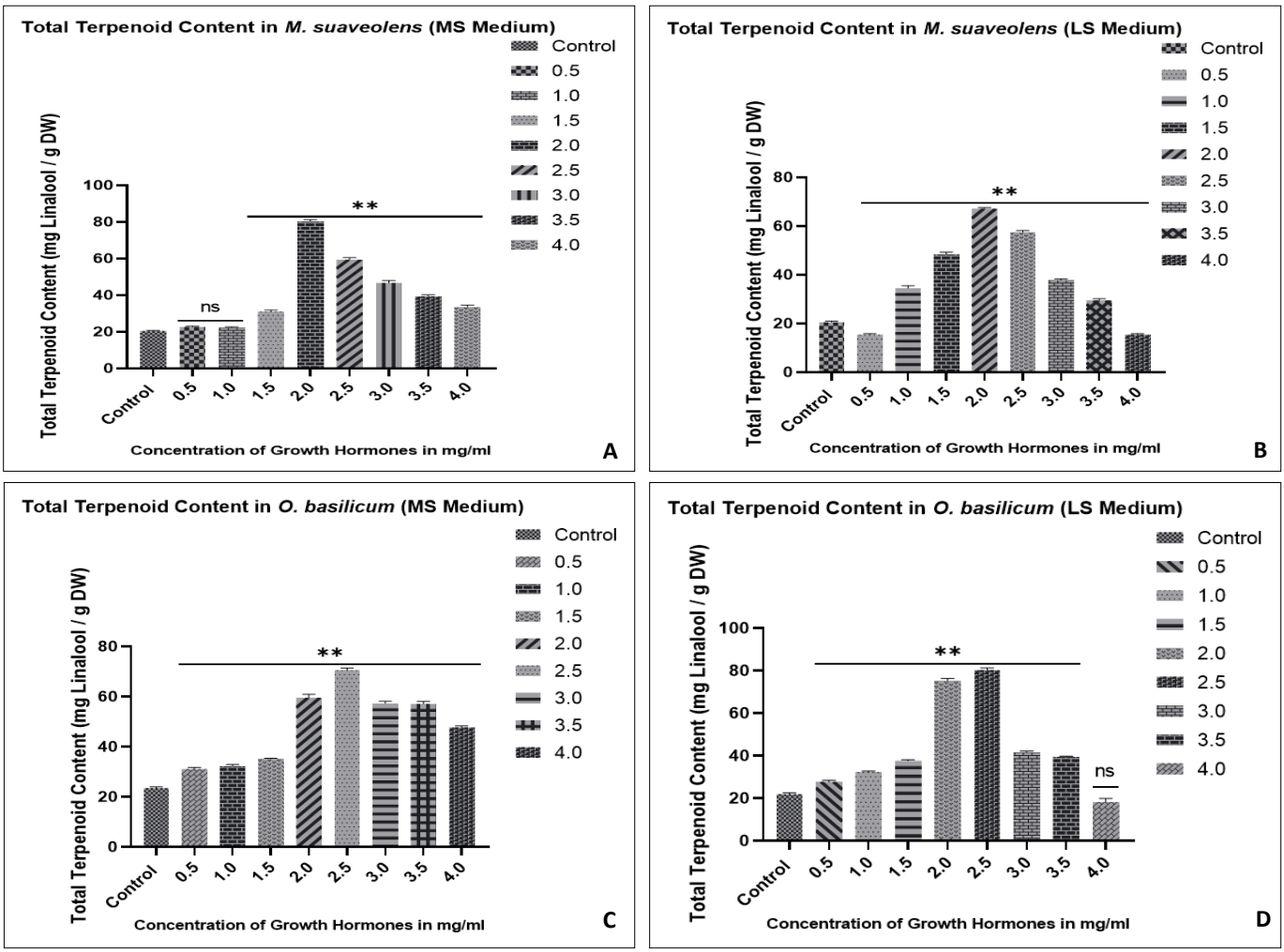

Fig. 9. Total Terpenoid Content in **A**. *Mesosphaerum suaveolens* grown on MS Medium **B**. *Mesosphaerum suaveolens* grown on LS Medium **C**. *Ocimum basilicum* grown on MS Medium and **D**. *Ocimum basilicum* grown on LS Medium where ns represents non-significant value and **p<0.01.

was significant growth of both plants, there was a concomitant increase in the levels of phenolics, flavonoids, and terpenoids. On comparing both plants, it was seen that the level of 2.0 mg/mL of NAA and BAP in combination showed the highest TPC at a concentration of 17.58 ± 0.32 mg GAE/ g DW, TFC at a concentration of 11516 ± 176.1 mg Quercetin/ g DW, TTC at a concentration of 80.25 ± 1.183 mg Linalool/ g DW in *M. suaveolens,* and 2.5 mg/mL of NAA and BAP in combination showed the highest phenolics at a concentration of 15.79 ± 0.13 mg GAE/ g DW, flavonoids at a concentration of 9513 ± 68.41 mg Quercetin/ g DW and terpenoids at a concentration of 75 ± 1.093 mg Linalool/ g DW in *O. basilicum.* It was found that total phenolics and total terpenoids in *O. basilicum* were higher than those in *M. suaveolens* whereas the level of total flavonoids was found to be higher in *M. suaveolens* compared to *O. basilicum.* Thus, the study opens new avenues for elucidating the role of NAA and BAP in biotransformation studies and hence, can be used to increase the yield of other important metabolites of industrial importance.

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Authors' contributions

RM has done the experimental work, including methodology, analysis, interpretations and writing the manuscript. KR has helped in preliminary plant collection, identification and herbarium preparation. PP has helped in the review and editing of the manuscript. MK has done conceptualization of the proposed work and method validation.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests to declare.

Ethical issues: None.

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