Effect of calliterpenone on growth, herb yield and oil quality of *Mentha arvensis*

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**Abstract**

Calliterpenone, a phyllocladane diterpenoid isolated from the leaves of *Callicarpa macrophylla* Vahl. (Verbenaceae) was found to be an excellent plant growth promoter. Its effect was observed in *Mentha arvensis*, which is a source of commercial menthol. Calliterpenone induced quick sprouting, more growth, branching and herb yield in the plant thereby increasing the production of the essential oil and menthol. At some concentrations, effect of calliterpenone was found to be even better than GA$_3$. Sowing of 72 hours pre treated suckers of *M. arvensis* in 0.1 mM solution of calliterpenone took lesser time for initiation of sprouting and produced 75 & 133% more numbers of sprouts which attended 33 & 77% more growth in height as compared to GA$_3$ and control respectively. Calliterpenone produced 12-25% more numbers of leaves per sprout as compared to control however, maximum numbers of leaves were recorded in 0.001 mM GA$_3$ treated suckers. Maximum size of the leaves were found in 0.001 mM calliterpenone treated suckers having 42% and 54% more leaf area as compared to GA$_3$ and control respectively. At 100 days of plant growth only foliar application of either GA$_3$ or calliterpenone at 15 days intervals enhanced height and herb yield per plant as compared to control but both soaking of suckers and spraying of sprouts with 0.001mM calliterpenone exhibited 100% & 50% more herb yield than the control and GA$_3$ respectively. Oil content varied from 0.8 - 1.1% in various treatments with 77.1% & 85.5% menthol contents as compared to 0.8% and 77.3% in control. Maximum menthol content 85.5% was recorded in 0.1 mM calliterpenone soaked and sprayed treatment. Although, spray of 0.1 mM solution of GA$_3$ at 15 days interval gave 5% more herb yield to that produced by 0.001mM calliterpenone pre-soaking of suckers and foliar spray treatment, the later treatment gave better results as far as oil content and menthol content in the oil are concerned, proving that this treatment is beneficial for *M. arvensis* cultivation.

**Keywords:** Calliterpenone, *Callicarpa macrophylla*, growth promoter, gibberellic acid, *Mentha arvensis*.

**INTRODUCTION**

Plant growth promoters hold commercial importance in intensive agriculture and have become quite valuable in agro-business of high value crops for organic cultivation. Auxins, gibberellins, cytokinins, abscisic acid, ethylene and many other classes of compounds have been reported to possess plant growth regulatory activities. Of all groups of compounds, gibberellins are most important (Srivastava, 1999). Various plant growth regulators have been found to enhance the herb and oil yield in mint species (Zlatev et al., 1977; Zlatev et al., 1978, Moghazy et al., 1979, El-Keltawi et al., 1987). Among them auxins, GA$_3$ (gibberellic acid) and cytokinins have been evaluated for enhancing the productivity of crops. In *M. arvensis*, GA$_3$ has been found to induce early sprouting and produce 30-50% enhancement in the yield of total herb with increase in internodes distance and leaf area by foliar spray at pre-flowering stage, (Kaul et al., 1965) enhancement in herbage and oil yield per hectare (Duhan et al., 1978), increase in menthol content in the oil of *M. piperita* vulgaris with foliar spray of IBA and gibberellic acid are also reported (Moghzay et al., 1979). Further, in *M. spicata* gibberellins and ethereal were found to increase the biomass fresh weight, leaf area, leaf weight, and chlorophyll content (Singh et al., 2001). The high cost of plant growth promoters restrict their application to high value crops only.

Recently, phyllocladane diterpenoids isolated from the plant *Callicarpa macrophylla* Vahl. (Verbenaceae), have been reported to enhance seed germination, root growth and shoot growth in several herbaceous mono- and dicotyledonous plants. Among different phyllocladane diterpenoids isolated from the plant, calliterpenone (Fig. 1) has been found to be most
promising (Singh et al., 2004, Singh et al., 2005a). Calliterpenone and its mono acetate also induces significantly higher growth and multiplication of in vitro shoot cultures of *Rauvolfia serpentina* at 0.25 and 0.5 mg/L concentrations compared to other available plant growth regulators (0.1-5.0 mg/L) tested under in vitro conditions (Goel et al., 2007). The chemistry of calliterpenone is well documented (Chaterjee et al., 1972, Fujita et al., 1975, Agrawal et al., 1995, Agrawal et al., 1996).

**Figure 1:** Calliterpenone

In the present communication, effect of calliterpenone on the plant growth, herb and oil yield and menthol contents of oil in the plants of *Mentha arvensis* var. Kushal have been studied and compared with the effects produced by GA3 in similar conditions.

**MATERIALS AND METHODS**

Calliterpenone was isolated as per published procedure (Singh et al., 2007). Stock solutions of 0.1 mM calliterpenone and GA3 (Mol. wt. 320 and 346 gm/mol) were prepared by dissolving 3.2mg calliterpenone and 3.46mg GA3 separately in 1ml of absolute alcohol and pouring these solutions in 20ml of distilled water in a 100ml volumetric flask. The solutions were mixed thoroughly and the volume was made up to 100ml by adding distilled water. 10ml of these solutions were taken and the volume was made up to 100ml and 1000ml by adding distilled water to prepare 0.01mM and 0.001mM solutions, respectively of both the compounds. One liter of each solution was thus prepared and used as stock solutions for further experiments. Suckers of *Mentha arvensis* var. Kushal (Khanuja et al., 2006) were cut into 5cm long pieces bearing three nodes each. To record the effect of soaking of suckers (before sowing) on the sprouting and their growth, nine pieces (in replicates of 3) of these suckers were kept dipped in the solutions of calliterpenone and GA3 at 0.1, 0.01 and 0.001mM concentrations separately and kept for seventy two hours. One set of three suckers were also dipped in distilled water, which was used as control. The dipped suckers were taken out and sown in pre-prepared earthen pots using normal soil and FYM in the ratio 4:1. Thus, for each test solution, set of nine pots were used. One set of three pots was also sown using same type of soil and the suckers that were dipped in distilled water and this set was treated as control. Necessary maintenance was done using similar conditions in all treatments. Recording of observation in terms of number of sprouts, average height (cm), number of leaves, size of leaves (cm2) were taken on third and fifth day after sowing and there after an interval of five days till thirtieth day. Thereafter, three pots of each test solutions were separated. Solutions for foliar sprays of each dilution of both calliterpenone and GA3 were prepared and were applied to the separated sets as foliar spray at the interval of fifteen days. The plants of the first set were kept as such. Final observations on height of the plants, number of branches, fresh weight of the plants of both sets were recorded after 100th day of sowing and the plants were harvested on the same day. Average results of three replications of each sets were recorded. For recording oil yield and oil analysis pooled herbs of three replicates were utilized. Herb were hydro-distilled in a Clavenger’s type glass apparatus for three hours for estimation of essential oils and analysis of menthol content were done with the help of GC analysis as described (Singh et al. 2005b).

**RESULTS**

**Effect of soaking of *Mentha arvensis* suckers in different concentrations of calliterpenone on their sprouting and growth behavior**

**Effect on sprouting**

The suckers, which were kept 72hrs in different concentrations (0.1, 0.01, 0.001mM) of calliterpenone were planted in pots and were studied for their sprouting behavior for 30 days and the effect was also compared with GA3 under similar concentrations. The suckers, kept in distilled water for similar period were treated as control. The study revealed that the suckers kept in 0.1 and 0.01 mM concentrations of both calliterpenone and GA3, started sprouting early from third day after planting, while the suckers of control and low concentration (0.001mM ) of both the compounds exhibited late sprouting i.e. only on fifth day. In most of the calliterpenone treatments, sprouting was completed on tenth day after that there were no further increase in sprouts. In control also there was no increase in sprouting after the tenth day of planting. However, in GA3 at 0.01mM concentration, sprouting stopped early on fifth day while at 0.001mM, sprouting continued till fifteenth day. Calliterpenone exhibited 75% more sprouting than the control at 0.1mM concentration and as its concentration decreased, the number of sprouting also decreased. On the other hand, GA3 exhibited sprouting equivalent to control at 0.01mM while at 0.1 and 0.001 mM, sprouting was lower even than the control. This shows that
Calliterpenone is very effective in inducing sprouting in *M. arvensis* suckers at 0.1mM concentration even better than GA3 (Table 1 [Supplementary data]). However, at 0.01 and 0.001mM concentrations, it was not found very effective in inducing the sprouting.

**Effect on growth of sprouts**

Calliterpenone induced better growth in sprouts of *Mentha arvensis* at all concentrations than GA3 and control. However, it induced maximum growth in sprouts at 0.1mM concentration and as the concentration decreased, reduction in growth was observed. The least growth was observed at 0.001mM. The growth was 77, 52 and 35% more than the control at 0.1, 0.01 and 0.001mM concentrations respectively. On the other hand, in comparison to control GA3 exhibited maximum 33% more growth at 0.01mM concentration followed by 17% more growth at 0.001mM. This indicates that even at lower concentration, calliterpenone induces better growth than GA3 and at higher concentration (i.e. at 0.1mM) GA3 exhibited 66% less growth than calliterpenone at same concentration (Table 1).

**Effect on leaf production and leaf size**

Since the leaves of *Mentha arvensis* are the economically important part of the plant and is the source of mentha oil and menthol. The effect of calliterpenone was also observed on the production of leaves per sprout. The compound exhibited 12-25% more leaf production than the control. Maximum leaf production was observed at 0.1 and 0.01mM concentrations. GA3 at 0.01mM concentration produced 10% more leaves than calliterpenone at the same concentration. However, number of leaves at 0.1 and 0.001mM calliterpenone concentrations, was more than GA3 at same concentrations (Table 1).

Calliterpenone increased the size of leaves at all concentrations as compared to control. At 0.1 and 0.001mM concentrations treatments of calliterpenone, leaf sizes in the sprouts were even bigger than GA3 treatments at the similar concentrations. However at 0.01mM, GA3 produced even bigger leaves in the sprouts than calliterpenone (Table 1).

**Effect of calliterpenone on herb and oil yield of Mentha arvensis**

Maximum accumulation of menthol in *M. arvensis* is reported to be after 100 days of planting of suckers, after which crop should be harvested for extraction of oil6. More height, branching and herb yield results in more oil yield from the plant. For seeing the effect of calliterpenone on these parameters and to compare its effect with GA3, one group of the suckers were planted directly in pots with out any treatment, the second group was soaked in different concentration of calliterpenone and GA3 for 72 hrs. After sprouting, plants of all the groups were sprayed with different concentrations of calliterpenone and GA3 at the interval of 15 days. Control suckers and plants were not given any treatment. Plants were harvested after 100 days and the results are presented in Table 2 [Supplementary data].

**Effect on plant height**

In the first group, where suckers were planted without any treatment and the growing plants were sprayed with different concentrations of calliterpenone, it was observed that the spraying of higher concentration (0.1mM) of calliterpenone exhibited better growth in height than the plants that were sprayed with lower concentrations (0.01 and 0.001mM). On the other hand, after soaking the suckers of second group in different concentrations of calliterpenone and spraying after sprouting with same concentrations of calliterpenone, it was observed that at lower concentrations (0.001mM and 0.01mM), the growth in height of sprouts was better than at higher concentrations. In comparison with GA3, it was observed that when only spraying was done with calliterpenone on the sprouts, it exhibited better growth promoting activity than GA3 with the same treatments at all dilutions. However, the suckers and plants when treated with different concentrations of GA3 in the similar way as was done with calliterpenone, it was observed that GA3 induced better growth at 0.1 and 0.01 mM concentrations.

**Effect on branching**

It was observed that both soaking of suckers and or spraying of plants with different concentrations of calliterpenone or GA3 had effect on branching of the plant. However, only spraying of calliterpenone at the interval of 15 days has slightly better effect on the branching of the plants than the both soaking and spraying treatments. On spraying with 0.01mM concentration of calliterpenone, plants exhibited maximum branching followed by 0.001mM concentration. Least branching was observed when the growing plants were sprayed with 0.1mM calliterpenone. When the suckers were soaked in different concentrations of calliterpenone and its plants were also sprayed with the same concentrations, it was observed that at lowest concentration (0.001mM), plants exhibited maximum branching. Soaking and spraying with higher concentrations of calliterpenone retarded the branching in the plants.

When the branch inducing effect of calliterpenone was compared with GA3, it was observed that the spraying with 0.1mM concentration of GA3 induced more branching than calliterpenone, at the same dilution but in other lower dilutions calliterpenone performed better than GA3 revealing that calliterpenone induces more
number of branches at lower dilutions thus, comparable
effects to that produced by GA3 can be obtained with
the lower concentration of calliterpenone. But when
suckers were soaked in GA3 and the sprouts were also
sprayed with it, at 0.1 mM & 0.01mM it exhibited
better branching than similar treatment with
calliterpenone but 2.5 times more branches were
recorded in the sprouts which developed from
calliterpenone soaked and sprayed with 0.001 mM
solution revealing that calliterpenone induces
maximum branching at lower dilution while higher
concentration become toxic . An overall observation
shows that only spraying is more effective in inducing
branching than soaking and spraying.

Effect on the herb yield
Fresh weight of the plant which is most important &
economical part of mint cultivation from which oil is
extracted was greatly influenced and enhancement in
the yields were recorded in all the treatments as
compared to control. Only spraying of calliterpenone
at 0.01mM exhibited 83% more herb yield than the
control, while both soaking and spraying of
calliterpenone at 0.001mM concentration, exhibited
100% increase in the herb yield. This effect was even
better than GA3 at these concentrations. However, at
0.1mM concentration only spraying and at 0.01mM
concentration both spraying and soaking of GA3
exhibited slightly better activity than calliterpenone at
all dilutions.

Effect on essential oil / menthol content
A little enhancement in oil content as compared to
control was recorded in almost all the treatments except
in spray of 0.1 mM GA3 in which it was similar to
control. Maximum oil content 1.2% as compared to
calliterpenone with similar treatment 1.0% and control
0.8% was recorded in 0.001 mM GA3 treatment
through both soaking of suckers and followed by foliar
spray but significant enhancement in menthol content
was recorded in 0.01 mM calliterpenone treatments
either only foliar spray or both (85.5 and 83.4%) as
compare to control and other treatments (77.3-78.8%)

DISCUSSION

*M. arvensis* is usually propagated vegetatively through
suckers and increase in the sprouting of the suckers is
an important parameter to increase the herb yield of
the plant. Therefore, first observations were made on the
sprouting behavior of suckers on soaking in different
concentrations of the compound for 72 hours.
Calliterpenone was observed to initiate quick sprouting
like GA3 (Kaul et al., 1965) and at 0.1mM
concentration it also induced 75 &133% more
sprouting in the suckers than the control and GA3.

However, at lower concentrations (i.e 0.01 and
0.001mM), it was not found very effective as compared
to control (Table 1). Further, at 30 days after planting
the compound was also observed to cause 13-25%
increase in the number of leaves in growing sprouts
than the control. At 0.01mM, GA3 induced more leaves
than calliterpenone. However, at 0.1 and 0.001mM,
number of leaves induced by calliterpenone was even
more than GA3(Table 1). Calliterpenone also increases
the leaf size at lower concentrations in the suckers that
were soaked in its different concentrations. At
0.001mM, it exhibited 42% and 54% bigger leaves than
the control and GA3(0.001mM) respectively (Table 1).

*M. arvensis* plants accumulate maximum menthol in
the oil at 100 days after the planting and the crop
should be harvested after this time (Singh et al., 1988).
Therefore, results were also recorded after 100 days of
growth. It was observed that the plants exhibit good
growth when the suckers were soaked in 0.001mM
concentration of calliterpenone and were also sprayed
with the same concentration at the interval 15 days
during the growth period. At this concentration, the
growth in height of the plants was even better than GA3.
It exhibited 67% and 33% more growth in height than
the control and GA3. However, at higher concentrations
GA3 produced better growth than calliterpenone.
Although spraying alone with both compounds have
enhanced plant growth and yield per plant as compared
to control but both soaking of suckers and spraying of
sprouts was found to produce even better results at the
lowest dilution tested in case of calliterpenone.
Spraying of GA3 at 0.1mM concentration produced best
herb yield per plant but almost comparable yield can be
obtained with calliterpenone soaking and spraying at
0.001mM dilution. Moreover if we compare the oil
contents, the calliterpenone treatment produced better
oil yield.

Calliterpenone has been found very effective at lower
concentrations on inducing branching. Although it
induced maximum branching only on spraying at
0.01mM but it was also found better than GA3 and
control at 0.001mM concentration. However, retarding
effect on branching was observed on both soaking and
spraying the suckers at higher concentrations with
calliterpenone. But at 0.001mM, calliterpenone
exhibited better branching than GA3. Since,
calliterpenone was observed to enhance both growth
and branching in the plants of *M. arvensis*, therefore, it
also helped in the higher production of herbal mass
which is very important for oil yield from the plant.
Since, oil glands are located in the aerial parts of the
plant, higher herbal mass ensures more production of
oil. On spraying alone, calliterpenone at 0.001mM
shown to enhance 66% more herbage than the control
and from GA3 it exhibited 13% more herbage. However,
on soaking and spraying, at 0.001mM calliterpenone
exhibited 100% and 50% more herb yield than the
control and GA3, respectively. Calliterpenone was also observed to increase the oil yield and menthol content marginally at 0.001mM than the control. But its effect was slightly lower than GA3.

The study, therefore, shows that, Mentha arvensis suckers when treated with calliterpenone exhibit, quick-profuse sprouting, more growth, branching, bigger and more number of leaves which causes higher yield of herbage and oil. Menthol content also increased with its application. Therefore, it also enhanced the quality of oil. Various plant growth regulators have been found to enhance the herb and oil yield significantly in many mint species including M. arvensis(Moghazy et al., 1979; Kaul et al., 1965; Duhan et al., 1978; Singh et al., 2001) but their commercial use is not in practice due to high cost of PGRs. Calliterpenone which is isolated from cultivable plant Callicarpa macrophylla and complete package technology is also available to produce the ready to use formulation at commercial scale may prove as boon for agribusiness to increase the productivity of commercial crops.

References


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