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A study on the effects of methyl jasmonate on morphological traits of *Hypericum perforatum* using leaf and stem explants

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Abstract

This study examines how methyl jasmonate (MeJA) as chemical elicitor on MS medium affects the morphological traits of a plant (i.e. number of leaves, stem length, number of roots, root length and number of buds) using different explants in vitro over a period of 42 days. The explants consisted of leaves and stems from seedlings cultured on MS medium under sterile conditions. They were transferred to the MS medium containing methyl jasmonate as elicitor at several concentrations including control, 50, 100, 150, 250 and 500 µmol, at temperature of 3±21 °C and 16:8 hours light/dark per day. This research was conducted as a factorial experiment based on Randomized Complete Block Design (RCBD) through 3 replications with 8 explants each. The data analysis at significant level of 1% suggested that methyl jasmonate as elicitor affects the entire morphological traits under study. The highest number of leaves, stem length and number of buds at 250 µmol of methyl jasmonate were on average 3.56, 1.38 and 1.53 cm, respectively. Furthermore, the highest mean of stem length (1.58 cm) and number of roots (1.15) were observed in the leaves explant at 500 µmol of methyl jasmonate. Therefore, it is concluded that methyl jasmonate on the traits affected and leaf explants were the best explants.

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Introduction

Jasmonate acid (JA) and methyl jasmonate (MeJA) constitute a new family of plant hormones generally called the jasmonates, which play a key role in regulation of developmental processes (Gao *et al.*, 2004). Two decades after the initial discovery, the first physiological effects of jasmonate acid and methyl jasmonate were identified. These substances were then recognized as aging, growth-inhibiting and elicitor for secondary metabolism in different plant species (Balby and Doto, 2008). Typically, the external methyl jasmonate is used in plant cell culture for activating secondary metabolism. However, studies conducted on the effects of MeJA on how *hypericum perforatum* grows have indicated that jasmonates have a variety of biological functions such as growth and germination inhibition in seeds and pollens as well as growth inhibition in roots and photosynthetic systems (Rosatto *et al.*, 2002). The physiological effects of jasmonates on plants depend on the species, developmental stage, type of MeJA and the applied concentration (Martin *et al.*, 2002). Methyl jasmonate stimulates ethylene biosynthesis by enhancing the activities of ACC synthase and ACC oxidase, but its effect depends on the plant species and stage of evolution (Sambedner and Partier, 1993). In examination of how methyl jasmonate affects artichoke (*Cynara scolymus L.*) at 4 levels including 1.22, 2.43 and 4.88 nmol, it was found out that the lowest concentration of methyl jasmonate (1.22 nmol) significantly raised the plant height, leaf area, leaf weight and length, fresh and dry weights of the root system length, while the highest concentration of methyl jasmonate (4.88 nmol) reduced the leaf fresh and dry weights, root system length and plant height. These findings suggest that the methyl jasmonate hormone is more effective at lower concentrations, specifically influencing the root system (Colzes *et al.*, 1999). In an evaluation over the effect of methyl jasmonate and salinity stress on certain morphological traits and functions of German chamomile (*Matricaria chamomilla L.*), it was reported that the applied factors significantly contributed to the entire traits. The highest leaf area (LA), shoot and root dry weights and root dry weight and minimum leaf specific weight and root length were associated with plants sprayed at with 75 µmol of methyl jasmonate, observed at salinity of 6 and 14 dS/meter, respectively. At all levels of methyl jasmonate, the root dry weight in the treated plants was higher as compared to the control. The application of methyl jasmonate reduced the root longitudinal growth while increasing its weight (Salimi and Shekari, 2011). Regarded as natural reserves in the world, herbs provide beneficial medication for humans. The increasing uses of synthetic drugs substantially cause acute medical conditions such as autoimmunity due to frequent use as well as side effects of some drugs (Ghasemi, 2009). *Hypericum perforatum* is known as an extremely valuable plant belonging to the family Hypericaceae (Ghasemi Pirbaluti *et al.*, 2013). In Persian references, *hypericum perforatum* is known as "Gol-e Raee" and a few other names (Ghasemi Pirbaluti *et al.*, 2013). Due to its high adaptability to environmental conditions, *hypericum perforatum* has been widely spread across Asia, Europe and North America. This perennial plant species is frost-resistant, helliophyta and calciphile, usually growing in ecosystems such as roadsides, meadows and pastures (Kosuth *et al.*, 2003 ; Deltito and Bayer, 1998). The essential substance in this plant is hypercin (Bombardly, 1995). *Hypericum perforatum* has a variety of pharmacological effects including antinociceptive, anti-inflammatory, antitumor, antioxidant, antibacterial, antimicrobial and anticancer (Rezaie *et al.*, 2010). Moreover, great attention has been shifted to this plant for HIV/AIDS treatment (Bombardly, 1995 ; Kozlowski *et al.*, 1999). In addition to the pharmaceutical and medical applications in the self-care and cosmetics industry, these substances are frequently used in the food processing industry due to good antimicrobial properties as preservative and protective. Moreover, they have been recognized particularly for antioxidant and antimicrobial properties (Bairu *et al.*, 2007 ; Crockett, 2010). This study intends to evaluate the effect of methyl jasmonate as a chemical elicitor on plant morphological traits (i.e. number of leaves, stem, number of stems, stem size, root length, number of roots, number of buds) and various explants (leaves and stems) on the MS medium containing methyl jasmonate as elicitor *in vitro* as well as selection of the best treatment and explant in terms of effectiveness on the examined traits.

Materials and methods

In order to examine the role of methyl jasmonate as elicitor on the morphological traits under study, the *hypericum perforatum* seeds were first supplied by Pakan Bazr Co., an Isfahan-based company in Iran. The seeds were then transferred to a tissue culture laboratory at the University, where they were kept in paper bags at room temperature (20-25 C° in dark). This research was done at the Agriculture Tissue Culture Lab, Islamic Azad University, Damghan Branch during 2013-2014.

A: Disinfection of seeds

The seeds are rinsed with water, then with a drop of dishwashing liquid in 70cc have shaken distilled water and then washed with distilled water and once we seed with sodium hypochlorite 1% (bleach) move in the chamber of the laminar hood we again with sterile distilled water 3 times with different time intervals under laminar hood washing machine we chamber (Pourshafee Anaraki, 2000).

B: Seeds of plants cultivated Hypericum and concentrations used

At the next stage, the seeds were cultured on MS (Murashige and Skoog, 1962) medium and then the test tubes were transferred to the growth chamber. In order to evaluate the effect of methyl jasmonate as elicitor on the plant morphological traits (i.e. number of leaves, number of stems, stem size, root length, number of roots, number of buds) and different explants (leaf and stem) on MS medium containing methyl jasmonate as elicitor in vitro. Several control concentrations of MeJA as elicitor including 50, 100, 150, 250 and 500 µmol) were applied for treating the plants.

C: How to prepare stimulus methyl jasmonate

The methyl jasmonate elicitor is an oily liquid, where 0.15 grams of MeJA was first weighted, and then poured and shook in 5 ml until it completely dissolved. Having filter-sterilized (0.2 µm) through the Laminar Flow Hood, it was added to the MS culture mediums containing different MeJA concentrations, which were then transferred into vials.

The mentioned treatments were carried out in triplicate each repeated with 8 explants. Within 60 days, the fully grown explants were isolated from various sections of the plant (stem and upper end of four or six-petals) and then transferred to culture mediums containing different concentrations of MeJA elicitor so as to examine the morphological traits from the base culture medium under laboratory conditions. Subsequently, the sample-containing tubes were transferred into the growth chamber at 23 C°.

D: Traits evaluation and statistical analysis

Throughout the eliciting treatments within 42 days, the measured traits included the number of leaves, stem length, number of roots, length root and number of lateral buds.

The data obtained by the RCBD-based factorial experiment were analyzed through SAS (SAS 9.2). Moreover, the mean values were compared through Duncan's test at 5% level. Finally, MS Excel was employed to draw statistical graphs.

Results and discussion

A: Effect of methyl jasmonate, time and explants on morphological traits

The table 1 shows the variance analysis results revealed that the effect of MeJA chemical elicitor and time was significant on the entire morphological traits under study (P=1%). Furthermore, there was a significant difference observed in examination of the explant effect on stem length at 1% as well as on root length at 5%.

Table 1. Analysis of variance effect of methyl jasmonate,Time and the explants on all the studied attributes.

		Ms				
Sov	.Df	number leaf	length of stem	number root	length of root	number sprouts
Explant	1	0.070 ^{ns}	3.102**	0.057 ^{ns}	0.128*	0.079 ^{ns}
Methyl jasmonate	5	2.035**	0.765**	0.213**	0.781**	0.945**
Time	5	137.232**	15.910**	19.099**	29.124**	13.041**
Methyl jasmonate *Explant	5	0.983**	0.232**	0.190**	0.061*	0.229**
Explant *Time	5	1.225**	0.003 ^{ns}	0.005 ^{ns}	0.015	0.022 ^{ns}
Metyhl jasmonate *Time	25	0.144**	0.050**	0.015 ^{ns}	0.069**	0.059**
Methyl jasmonate *Time* Explant	25	0.078*	0.018 ^{ns}	0.015 ^{ns}	0.012 ^{ns}	0.022 ^{ns}
Error	144	0.049	0.018	0.029	0.025	0.021

Ns,*,**:non significant, significant at p<0.05 and p<0.01, respectively.

B: Main effects of methyl jasmonate, time and explants

The table 2 for comparing the main mean effect of MeJA, time and explant on the traits of *Hypericum perforatum* indicated that the longest stem belonged to the explant with mean leaf of 1.24 cm, while the shortest belonged to the explant with mean stem of 1 cm. Moreover, the longest and shortest roots were observed in the case of leaf and stem samples with 1.39 cm and 1.34 cm, respectively, while the other traits showed no significant difference. In evaluation of the MeJA chemical elicitor, the highest and lowest mean number of leaves were observed at the 250 µmol treatment and control (zero) with 3.47 cm and 2.92 cm, respectively. As for the effect of MeJA on stem length, the longest and shortest mean growths were in the 250 µmol treatment (1.24 cm) and control (0.87 cm), respectively. As for the number of roots, the highest mean belonged to the 500 µmol treatment, while the lowest mean belonged to the 50 µmol treatment. Furthermore, the longest and shortest mean roots belonged to the 500 µmol and 50 µmol treatments with 1.57 cm and 1.21 cm, respectively. The highest number of buds were observed in the 250 µmol (mean of 1.09 cm) while the lowest belonged to the control treatment (mean of 0.66 cm), as illustrated in Table 2.

Table 2. Comparison of the effects of methyl jasmonate, and the explants on all the studied attributes.

	Number leaf		length of stem (Cm)		number root		length of root (Cm)		number sprouts	
The main effect	Mein and St.d	Grouping	Mein and St.d	Grouping	Mein and St.d	Grouping	Mein and St.d	Grouping	Mein and St.d	Grouping
Explants										
Leaf	3.25±1.66	a	1.24±0.63	a	1.34±0.7	a	1.39±0.87	a	0.91±0.59	a
Stem	3.29±1.99	a	1±0.65	b	1.31±0.69	a	1.34±0.84	b	0.95±0.6	a
Methyl jasmonate										
0	2.92±1.65	b	0.87±0.6	c	1.26±0.69	c	1.25±0.81	c	0.66±0.47	d
50	3.01±1.8	b	1.05±0.63	b	1.25±0.65	c	1.21±0.74	c	0.83±0.53	c
100	3.43±1.93	a	1.09±0.61	b	1.28±0.66	bc	1.27±0.76	c	0.94±0.55	b
150	3.39±1.87	a	1.22±0.69	a	1.35±0.69	ab	1.43±0.88	b	1.03±0.64	a
250	3.47±1.88	a	1.24±0.69	a	1.41±0.74	a	1.48±0.93	b	1.09±0.66	a
500	3.41±1.9	a	1.23±0.65	a	1.43±0.73	a	1.57±0.96	a	1.05±0.64	a
Time (week)										
7	0.2±0.22	f	0.13±0.13	f	0±0	f	0±0	f	0±0	f
14	1.61±0.35	e	0.57±0.2	e	1.07±0.16	e	0.53±0.08	e	0.45±0.17	e
21	3.62±0.49	d	1.06±0.29	d	1.39±0.22	d	1.53±0.27	d	0.95±0.26	d
28	4.27±0.44	c	1.46±0.26	c	1.71±0.21	c	1.87±0.24	c	1.21±0.26	c
35	4.85±0.42	b	1.65±0.25	b	1.82±0.2	b	2.05±0.26	b	1.44±0.27	b
42	5.08±0.43	a	1.84±0.29	a	1.99±0.2	a	2.23±0.29	a	1.55±0.28	a

Means in each column with the same letters are not significantly different at 5% level.

Moreover, the valuation of the time effect on morphological traits, the table for comparison of mean values demonstrated that the longest and shortest traits under study took 42 and 7 days, respectively.

C: Interaction of methyl jasmonate and explant

The results of Fig. 1 showed that as for the number of leaves, the highest mean belonged to the stem explant in 100 µmol treatment (3.56 cm) while the lowest in mean was observed in the stem explant and control treatment (2.69 cm). As shown in Fig. 2, regarding the stem length, the highest and lowest means belonged to the leaf explant at 100 µmol treatment (1.38 cm) and stem explant at the control treatment (0.62 cm), respectively.

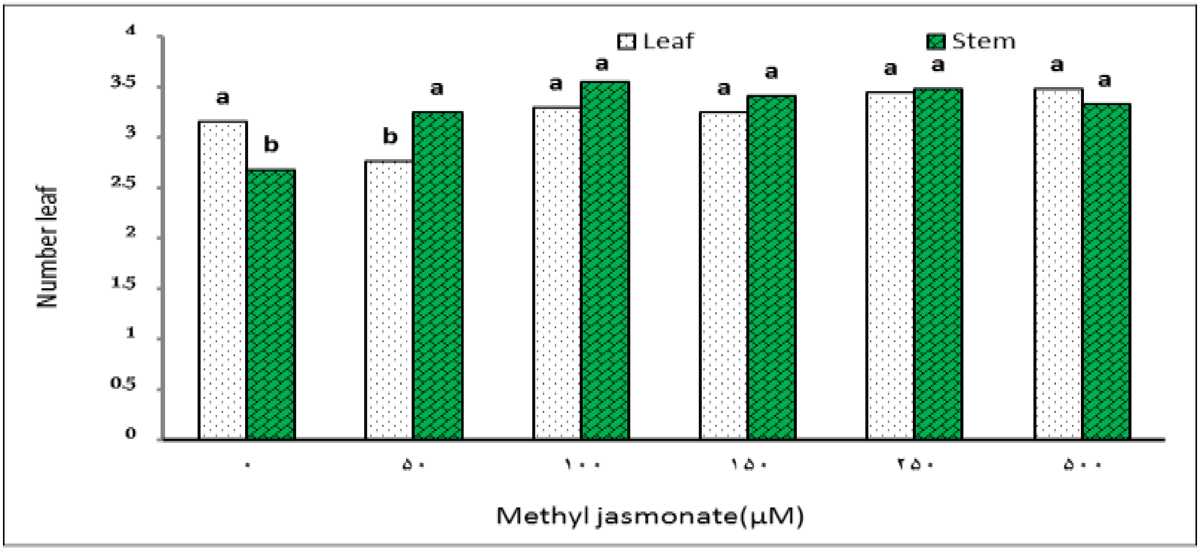

Fig. 1. The interaction of methyl jasmonate and explants on Number leaf.

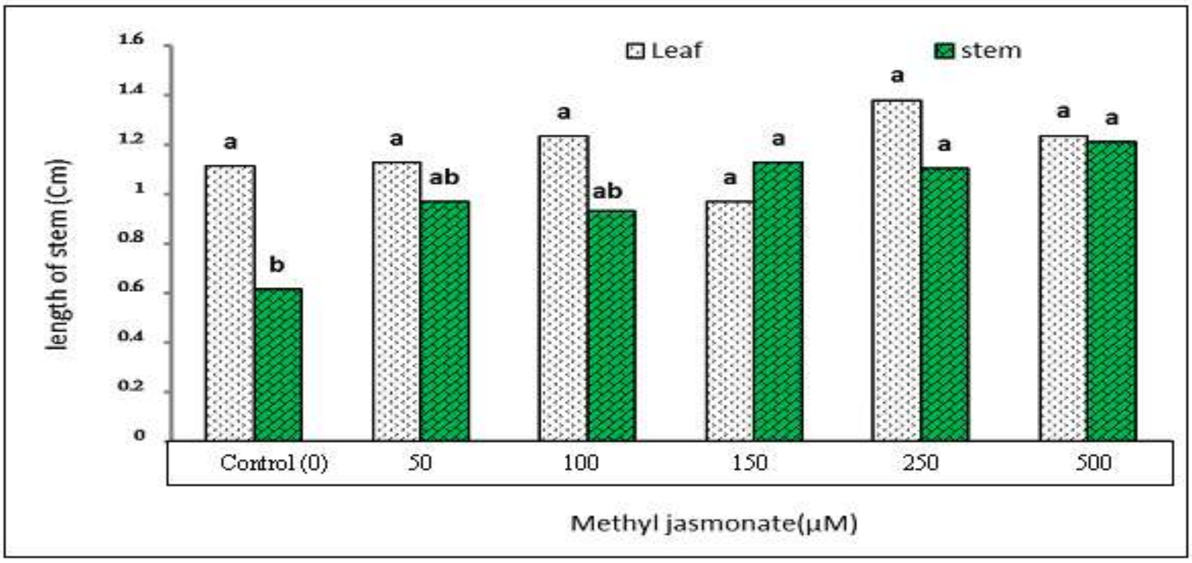

Fig. 2. The interaction of methyl jasmonate and explants on length of stem.

Fig. 3 shows the highest mean number of roots was observed in the leaf explant at the 250 µmol treatment (1.53 cm) while the lowest mean belonged to the stem explant and the control (1.19 cm) as illustrated in. As for the root length, the results of Fig. 4 showed that the highest and the lowest means belonged to the stem explant at 500 μmol treatment (1.58 cm) and the stem explant at 50 μmol treatment (1.17 cm).

The results of Fig. 5 indicated that as for the number of buds, the highest mean number of buds belonged to the leaf explant at 500 μmol treatment (1.15 cm) while the lowest was observed in the leaf explant and the control (0.53 cm).

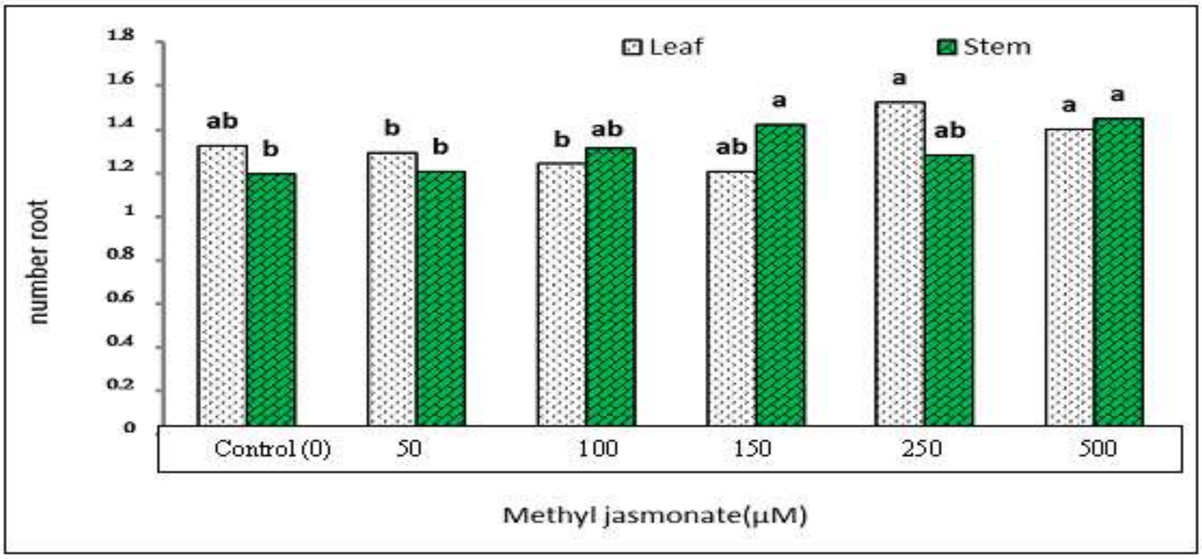

Fig. 3. The interaction of methyl jasmonate and explants on number root.

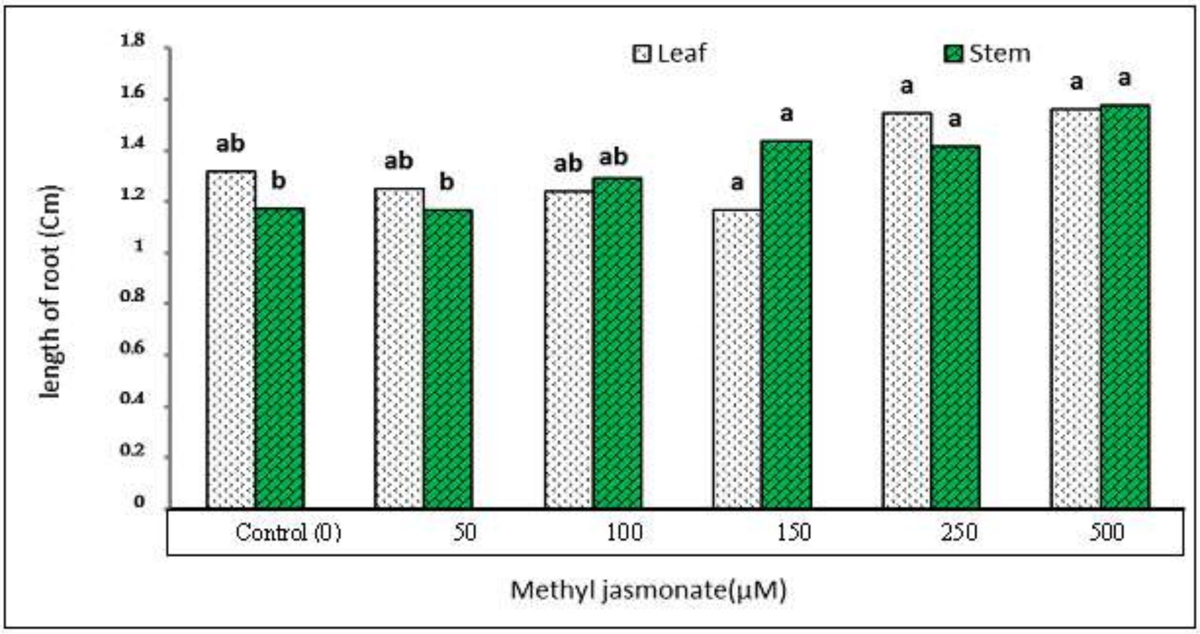

Fig. 4. The interaction of methyl jasmonate and explants on length of root.

D: Interaction of explant and time

The Fig. 6 indicated that as for the number of leaves, the highest mean belonged to the stem explant within 42 days (5.25 leaves) while the lowest was observed in the stem explant within 7 days (zero leaves), while there was no significant effect in the case of other traits.

The results of this study suggested that methyl jasmonate as chemical elicitor affects the entire morphological traits of the plant. Based on the differences between MeJA treatments, it can be concluded that application of 250 µmol MeJA would lead to increased number of leaves, stem length and number of buds, while 500 µmol MeJA would lead to increased root length and number of roots. Moreover, it can be argued that the stem explant provided the best explant of all just as time desirably influenced the entire morphological traits.

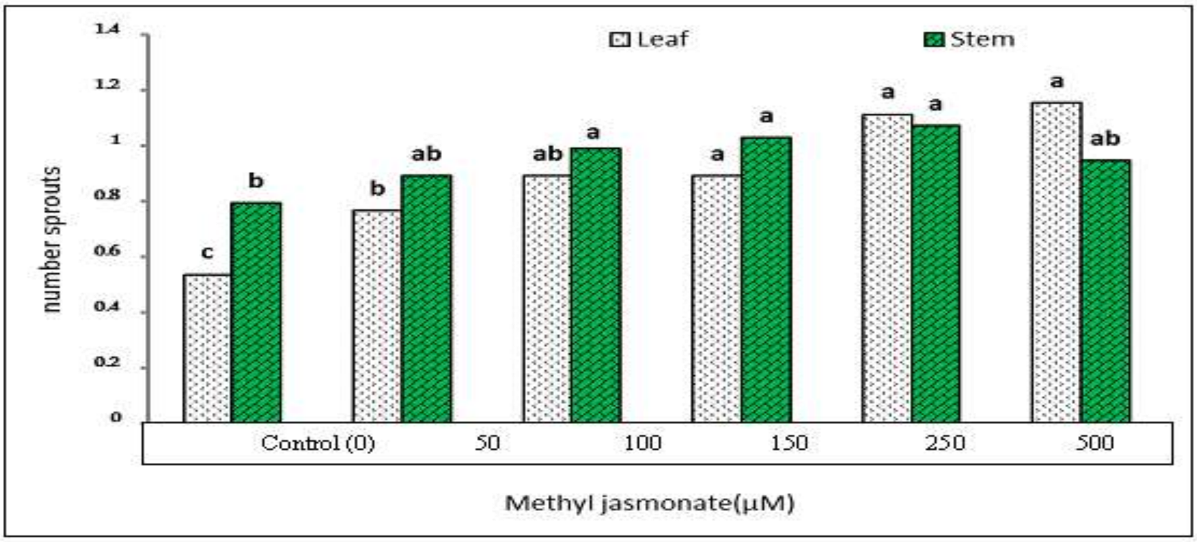

Fig. 5. The interaction of methyl jasmonate and explants on length of root.

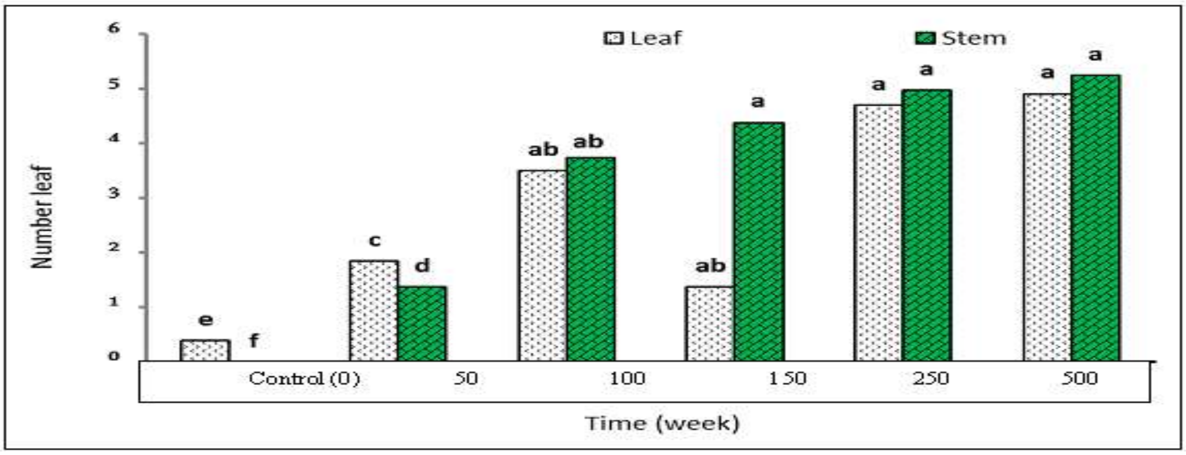

Fig. 6. The interaction of Explants and Time on length of on Number leaf.

Balby and Doto (2008) identified the physiological effects of MeJA as chemical elicitor, i.e. these substances were later recognized as aging, growth-inhibiting and elicitor for secondary metabolites in different plant species. Masijuska and Kobsevich (2002) reported that methyl jasmonate at concentrations higher than 10^{-3}M and 10^{-4}M reduces the growth of roots and stems in Pharbitis nil, while it leaves an elicitation effect on root and stem growth at concentration 10^{-4}M. In another study, Keramat and Danesh (2012) found out low concentrations of MeJA leads to increased chlorophyll level in soybean, while higher concentrations reduces the level of photosynthetic pigments. Choudhury and Panda (2004) reported that since methyl jasmonate can induce ROS production in plants, an effective antioxidant system under stress and non-stress conditions would be necessary to maintain metabolic functions.

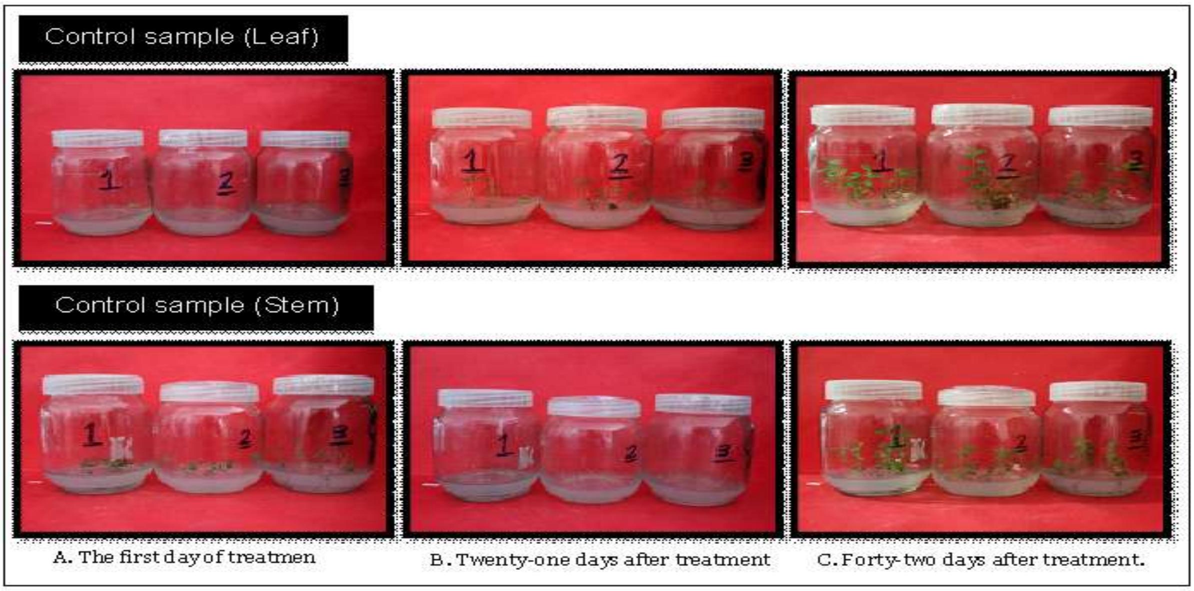

Fig. 7. Overview of the control sample (without any growth regulator) on *Hypericum*.

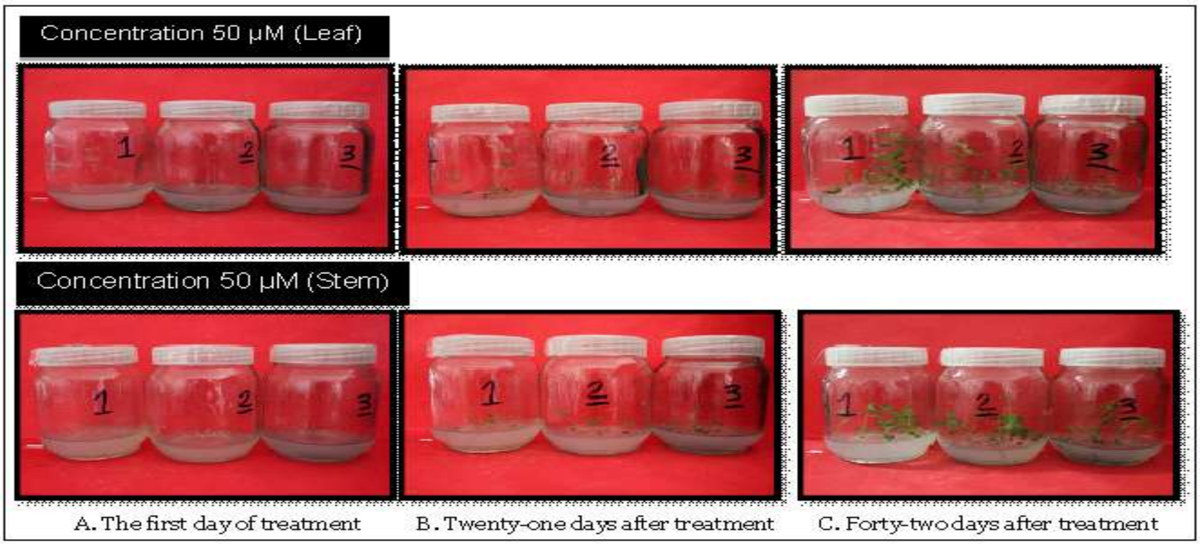

Fig. 8. Overview of the stimulus effect of methyl jasmonate on *Hypericum*.

The antioxidant systems in plants are divided into enzymatic and non-enzymatic. Zobayed *et al* (2005) The former covers superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), glutathione Reductase (GR) and peroxidase (POD), while the later covers ascorbate, glutathione, carotenoid, phenolic compounds and anthocyanins. All the concentrations of MeJA as elicitor applied in this study greatly affected the entire plant morphological traits. Cao *et al* (2009) demonstrated that MeJA treatment would significantly reduce the intensity of damage to marigold known as chilling injury caused by low temperatures, thus decreasing the activity of enzymes involved in lignin biosynthesis, i.e. PAL, POL and PPO.

Conclusion

The results of this study indicated that methyl jasmonate as chemical elicitor affects the entire morphological traits and enhanced the growth rate. Accordingly, it seems that higher concentrations of MeJA (250 and 500 µmol) leave higher impact on the growth parameters as compared to lower concentrations (control, 50, 100 and 150 µmol). Through application of MeJA treatment at 250 µmo, the number of leaves, stem length and number of buds experienced an increase by 3.56, 1.38 cm and 1.53, respectively. Moreover, the 500 µmol MeJA led to increased root length by mean of 1.58 cm, while the mean number of roots was 1.15. Similarly, time was found out to be an influential factor. Finally, it can be concluded that the leaves provided the most ideal explant of all.

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