

# Quantitative and Morphological Effect of Plant Growth Regulators on Callus Initiation from Leaves and Roots Explants of *Artemisia annua* L.

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**Abstract.** To study the effect of some plant growth regulators on the in vitro initiation of callus using the leaves and roots explants of *Artemisia annua*. The experiment was arranged in a completely randomized design with 2 replicates. Morphologically leaf explants culture in media having the composition MS Basal + NAA (2.0mg/l) + BA(1.0 mg/l) shows best results as compared to all other media. However in roots shows no growth in all the media composition. On the other hand media having a composition (MS Basal + 2,4-D(1.0mg/l) + Kn(0.1mg/l)) with growth hormone antagonist shows no callus formation neither in leaf or roots. Our studies are also supported by the HPLC results about the artemisinin content in leaf in media having the composition MS Basal + NAA (2.0mg/l) + BA(1.0 mg/l) is 0.052% and least in media a composition (MS Basal + 2,4-D(1.0mg/l) + Kn (0.1mg/l)) have only 0.012%. On the other hand, we didn't found any artemisinin content in the root explants in all the media. Our present study suggest that Benzyl adenine (BA) could be used as a promising growth hormone in tissue culture.

## 1. Introduction

Now a day's most of biologically active molecules with biological activities such as antimicrobial, insecticidal dyes, flavors, fragrances, pigments, agrochemicals and pharmaceuticals are extract from plants. To gets these product throughout the years with high productivity of secondary metabolite researchers develops a technology known as Plant cell culture (Dicosmo and Misawa 1995).

*Artemisia annua* L. (Asteraceae), is a sources of aromatic product is an annually growing herb, known as Sweet Wormwood is cultivated in China as a medicinal plant. Due to a source of aroma chemicals forms leaves of *Artemisia annua* used in fragrance industry this plant is now cultivating recently in Europe and India. Type of pollination is cross by either wind or air having diploid with chromosome number,  $2n=18$ . A portion of the naturally dynamic optional metabolites substantiate the case made in customary arrangement of prescription (Bhakuni *et al.* 2001).

Some exploration in the home grown medication prompts the improvement of new medication from *A. annua*, the wellspring of qinghaosu (artemisinin), an exacerbate that shows guarantee as an enemy of malarial specialist (Klayman 1985). Artemisinin likewise found as a plant inhibitor and herbicide (Pamela *et al.*, 2011). Artemisinin has been appeared to specifically murder malignant growth cells and human disease. In the ongoing investigation, the viability of a consolidated treatment of dihydroartemisinin (DHA artemisinin simple) and butyric corrosive at low portions in murdering malignancy cells offered a less dangerous, modest, and successful disease chemotherapy. There have been energizing preclinical outcomes against leukemia (Mueller *et al.* 2004) and other disease cells.

The business wellsprings of most artemisinin are from field developed leaves and blossoming highest points of *A. annua*, which are exposed to regular and physical variety and pervasion of microorganisms, organisms, and bugs that can influence the utilitarian therapeutic substance of this plant (Klayman 1985). Constrained accessibility from enormous scale trimming of *A. annua* plants

combined with extreme interest for the restorative has prodded researchers to investigate substitute strategies for generation.

Plant cell culture isn't influenced by changes in natural conditions, so improved generation might be accessible in wherever or season. A choice to field-developed harvests or to concoction amalgamation has been enormous scale in vitro plant tissue culture (Francois et al. 1990; Dhingra et al. 2000). Roots and buds are initiated by callus from poplar stems (Simon EF, 1990). White prompted callus got from tumor-creating procambial tissues of half breed *Nicotiana* that did not require hormone supplementation. Gautheret (1939) and Nobecourt (1939) had the option to keep up callus societies of carrot utilizing auxin hormone increases.

The Chinese antimalarial tranquilize artemisinin (Qinghaous, QUS) is one such new medication of current intrigue. Artemisinin has quick activity and is compelling against chloroquine-safe malarial parasites and has an alternate method of activity from existing blood schizonticides (Nair et al.1986). Artemisinin (qinghaosu) is a metabolite of *Artemisia annua* L, go about as a sesquiterpene lactone with antimalarial safe against *Plasmodium* spp. and flow inquire about additionally demonstrates that artemisinin medications are viable against disease (Efferth et al 2001), *Leishmania* (Yang and Liew, 1993; Sen et al., 2007), *Trypanosoma* (Mishina et al., 2007), and some infections (Romero MR 2005). The high antioxidative movement of *A. annua* is because of high substance of flavonoid. Likewise, *A. annua* leaves and rough concentrates have been accounted for to be a decent wellspring of cell reinforcements (Cai et al., 2004), being among the four therapeutic plants with the most elevated ORAC (oxygen radical absorbance limit) level among different medicinals (Zheng and Wang, 2001). High cancer prevention agent movement is because of the high substance of leaf flavonoids (Bilia et al., 2006), this might be credited to the newfound C-glycosyl flavonoids as a perhaps in charge of antioxidative and antiviral action (Han et al., 2008).

## 2. Material and Methods

Studies were carried out in the Department of Biotechnology, Jamia Hamdard, Delhi, India. The explants were sterilized and incubated on Murashige and Skoog basal media (MS medium, 1962). augmented with concentrations 0.5 to 1.0 mg/l of kinetin (kn), Benzyl adenine (BA), 2,4-Dichloro Phenoxy Acetic Acid (2,4-D).

**INOCULATION AND MAINTENANCE OF CULTURES:** Callus cultures were initiated by transferring different explants (leaf, petiole, intermodal, nodal segments and roots) of 30 days old *in vitro* grown plants of *Artemisia annua*, on different callus initiation media with various growth regulators. The compositions of media used for callus induction and their effects on different explants are listed in Table -1. The experiments were carried out in duplicate. The cultures were maintained at 25 °C and 16/8 Hr. light/dark regime. The cultures were maintained for 45 days and observations were recorded after every 5 days interval. After 45 days of culture, the artemisinin content (%) in each of the callus lines induced by various explants on different media was analysed by HPLC. Here we will show the results of only leaf and root explants Table -1.

**Table 1:** List of various media enhanced with different development controllers utilized for callus enlistment

Media	Composition
1	MS Basal+NAA(2.0mg/l)+Kn(0.5mg/l)
2	MS Basal+NAA(2.0mg/l)+Kn(1.0mg/l)
3	MS Basal+NAA(2.0mg/l)+BA(0.5mg/l)
4	MS Basal+NAA(2.0mg/l)+BA(1.0mg/l)
5	MS Basal+2,4-D(1.0mg/l)+Kn(0.1mg/l)

NAA - Naphthalene Acetic Acid, Kn – Kinetin, mg – milligram, BA - Benzyl adenine, 2,4-D - 2,4-Dichloro Phenoxy Acetic Acid, mg/L –milligrams per liter.

**High Performance Liquid Chromatography Analysis:** To break down the substance of artemisinin in the callus societies, the way of life were gathered and air dried altogether. These examples were gauged and put away. 1 gm dried example was powdered and removed with hexane (10ml X 3) at 400 C, with the difference in hexane after each 24 Hrs. The hexane parts were pooled and focused under diminished weight. The buildup acquired was defatted with acetonitrile. The encouraged fat was sifted through and the filtrate was focused under decreased weight. The buildup subsequently acquired was broken down in 2 ml methanol (MeOH). 20 µl aliquot from the arrangement was then utilized for making Q260, as artemisinin does not have any chromophore for UV discovery in HPLC and afterward it was settled unmistakably on HPLC. It was derivatized by first adding 3% NaOH to each example, brooding the example in shaking water shower at 5 °C for 30 min, cooling at 4 °C for 5 min. At that point include 1M GAA in 20 % MeOH and keep up the pH at 6.8 (Numonov et al., 2019).

**Location and Quantification Of Artemisinin:** Derivatized artemisinin (Q260) was settled through a switched stage section utilizing MeOH: 10mM phosphate support (pH 7.9) in the apportionment of 60:40 as the portable stage at a consistent stream rate of 1 ml with the identifier set at a wavelength of 260 nm utilizing HPLC hardware (Water Delta 600, USA).

### 3. Results

Among the various explants and media, leaf explants and Medium four with composition- ‘MS + Sucrose (30 g/L) + Agar (8 g/L) + NAA(2.0mg/l) + BA(1.0mg/l) and cultured at 25 °C and 16/8 hr light/dark regime were found best for callus induction and artemisinin content. The observations for different explants viz. leaf, petiole, node, internode and root inoculated on 10 different mediums were recorded after 5 days interval but we present results of only leaf and roots explants in day 0, 15, 25 and 45 in Table- 2 and 3.

**Table 2**

<i>Duration (days)</i>	<i>Leaf</i>				
	<b>Media 1</b>	<b>Media 2</b>	<b>Media 3</b>	<b>Media 4</b>	<b>Media 5</b>
0	Inoculation	Inoculation	Inoculation	Inoculation	Inoculation
15	Green in color with swelling	Yellowish green swelling	Little green yellow and swelling	Yellow, green swelling	Green Swelling
25	Greenish yellow in color with swelling	Same as above	Yellow green + swelling	Yellow callus	Green Swelling
45	Brown and Callus“ “	Yellow + Callus	Callus initiation + green	Light green callus	NO GROWTH

**Table 3**

<i>Duration (days)</i>	<i>Root</i>				
	<b>Media 1</b>	<b>Media 2</b>	<b>Media 3</b>	<b>Media 4</b>	<b>Media 5</b>
0	Inoculation	Inoculation	Inoculation	Inoculation	Inoculation
15	Greenish yellow with swelling	Yellow Green Swelling	Yellow, Brown + Swelling	Yellow Swelling	Yellow Swelling
25	Whitish Green + Callus	Greenish white with callus	Yellow & Brown Swelling	NO GROWTH	Yellow Swelling
45	Brownish Callus	Greenish yellow Callus	NO RESPONSE	NO GROWTH	NO RESPONSE



Leaf - 1



Leaf - 2



Leaf - 3

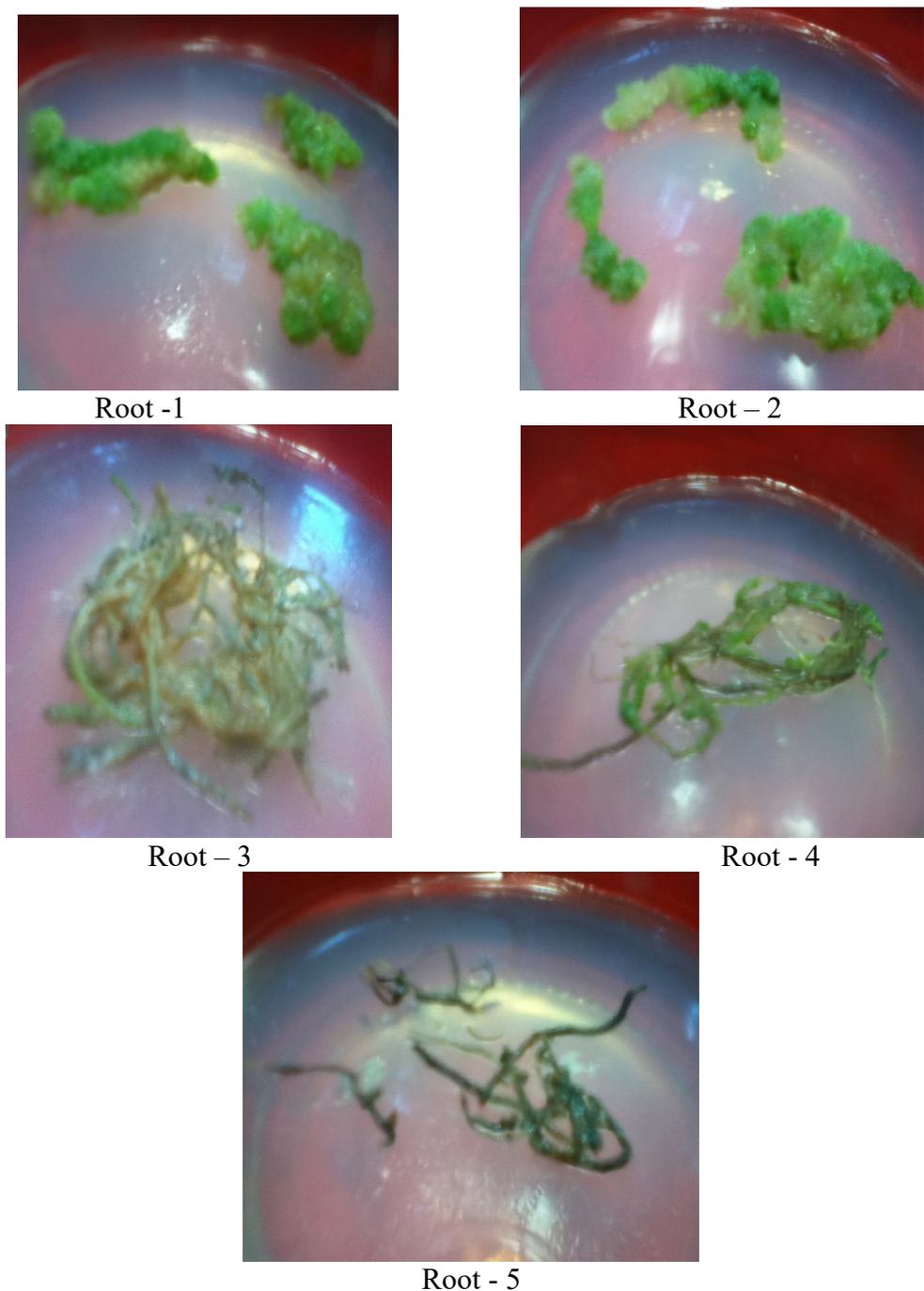


Leaf - 4



Leaf - 5

**Figure 1:** Leafs after 45 days of inoculation



**Figure 2:** Roots after 45 days of inoculation

As observed the highest artemisinin content was found in callus line induced from leaf explants on medium 4. The data is shown in table -4

**Table 4:** Depicting the artemisinin content in different callus cultures induced from various explants of *Artemisia annua* L. on different medium after 45 days of inoculation

Medium	Composition	Leaf	Root
1	MS Basal + NAA(2.0mg/l)+Kn (0.5mg/l)	0.028%	-
2	MS Basal + NAA(2.0mg/l)+Kn (1.0mg/l)	0.027%	-
3	MS Basal + NAA(2.0mg/l)+BA (0.5mg/l)	0.049%	-
4	MS Basal + NAA(2.0mg/l)+BA (1.0mg/l)	0.052%	-
5	MS Basal + 2,4-D(1.0mg/l)+Kn (0.1mg/l)	0.012%	-

#### 4. Discussion

Among the explants viz., leaf and root, it can be concluded that the leaf explants gave the best response in terms of growth (fig-1) and artemisinin content in almost all media. During observations, medium (MS Basal + NAA(2.0mg/l)+BA (1.0 mg/l)) was found best in terms of growth of callus from leaf explants. Callus initiated from leaf explants on medium number (MS Basal + NAA (2.0mg/l) + BA (1.0mg/l)) gave the highest artemisinin content of 0.052% whereas the lowest (0.012%) was found in callus initiated on medium (MS Basal + 2,4-D(1.0mg/l)+Kn (0.1mg/l)) (table-4). As evident from the observations, the hormonal interplay has a significant role in tissue culture. The choice of explants and the combination of hormones are the key factors influencing initiation of callus cultures. The study of growth pattern and increase in secondary metabolite content with respect to growth in callus lines is very essential. The ratio of increase in biomass and increase in artemisinin content with respect to time would be highly useful in further studies. This study will help in increasing the artemisinin production through callus culture by optimising the culture conditions. This can further be utilized for developing suspension cultures. The yield enhancement strategies, like the use of compounds such as elicitors, growth regulators, precursors and permeabilising agents may further be employed for increased artemisinin production. The study of artemisinin content with respect to different explants has been crucial in screening out explants and media with better expression of artemisinin.

Almost all the explants, except root (fig-2), showed growth on various media. The leaf explants (fig-1) gave the best response in media number four and some growth in all other media in terms of callus initiation as well as artemisinin content. As cited in literature, the commercial sources of most artemisinin are from field grown leaves and flowering tops of *A. annua*, this is also evident from our observations. The callus cultures initiated can further be exploited to develop cell suspension cultures. The suspension cultures can further be exploited to investigate the effects of various chemical factors on modulation of artemisinin production and to shed more light on regulation of artemisinin biosynthetic genes in response to specific compounds.

#### Conclusion

Most of biologically active molecules with biological activities such as antimicrobial, insecticidal dyes, flavors, fragrances, pigments, agrochemicals and pharmaceuticals are extracted from plants. But to cultivate these plants throughout the year is not possible naturally so overcome this now researchers are dependent on tissue culture. In present study a combined BA and NAA treatment is an efficient protocol for callus initiation and proliferation from explants using cultures of *A. annua*.

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