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Increasing of Some Secondary Metabolites Production in *Albizia lebbeck* Using Different Concentrations of Arginine *in Vitro*.

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ABSTRACT

Albizia lebbeck is considered one of medical plants and current study aimed to increase of budmunchiamine compounds by induction of callus from the Cotyledons leaves of Albizia. seeds grown in laboratory by adding different levels of 2, 4-D and BA and the better combination of them was (2, 0.75) mg/L 2,4-D and BA respectively then the same concentration was prepared the MS medium in which the amino acid L-Arginine was added (0, 80, 160, 240, 320) mg /L. It lead to reduction the dry and fresh weight of callus with a significant increase in all compounds and Concentration 320 mg /L gave the highest amount of Budmunchiamine L5, Budmunchiamine L4, Budmunchiamine L2, Budmunchiamine L1 that amounted 65.78 , 57.75, 44.79, 79.16 $\mu g.ml^{-1}$ respectively from fresh callus compared to the control treatment, which gave the least amount of all Budmunchiamines compounds, whereas, the comparative treatment of callus showed the superiority of the active ingredients over the Albizia grown in the field.

Keywords: Albizia, Budmunchiamine, L- Arginin

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INTRODUCTION

During history, in his fight with illness, human has continually volunteered assist of herbals to opulent the health-elevating. ancients guides utilize was set from Nagpur on a Sumerian clay tablet about five thousand years old (Pham *et al*., 2019) However, the use of plants in handling disease is also aforesaid in of China 2500 BC and in addition to the ebers Papyrus Written in the Scripture Indian the year one thousand five hundred and fifty B.C (Petrovska, 2012), (Kumar *et al*., 2019). For thousands of years plants utilized in the protection and treatment of numerous human sickness. Nearly whole medicinal systems, Whether Traditional or European medicine, are founded from herbal-derived of medicinal value (Lu *et al*.,2016). Presently, there is a mounting desire to domain of natural proudacts ability curative impact against illness or preventive. (Zaynab *et al*., 2018) further analysis of the wealth hidden in plants. For what is known metabolic compounds it has very active function to protection from pathogens. (Liu *et al*., 2017), (Latif *et al*., 2017). *Albizia lebbeck* it is tree which belonging to the family of Leguminosae (Yadav *etal*.,2011) and used it in folk medicine to treatment of rheumatism, cough, diarrhea, wounds (Karuppannan *et al*.,2013) and from the leaves made a drink after boiled it and also the young shoots, root and bark are exceedingly utilized in folk medicine to prepare an infusion (hot or cold) to treat inflamed eyes and skin diseases such as scabies. Albizia genus it contains many medicinal compounds it has impact as bactericidal and antitumor like terpenes, saponins ,flavonoids , alkaloids, in addition to macro cyclic alkaloids (Ganguli and Bhatt,1993) like budmunchiamines L1- L3 isolated from Seeds (Varshney *et al*.,1976) and budmunchiamines L4 and L5 got it from leaves and stem bark its extract were used against malaria through to inhibit the malarial enzyme plasmepsin II (Ovenden *et al*.,2002) and because plants in nature produce these active substances, in very small quantities, the demand for them is not sufficient. Therefore, modern biological techniques have been resorted to (Kowalczyk *et al*., 2020) Plant tissue cultures at present utilized to agricultural purposes through clonal multiplication of plants (Hemanthakumar *et al*., 2019) and prouduce Callus cultures are often used successfully to more produce from metabolite *in vitro*. These proceedings have gived numerous promising outcomes that effective and low-cost sources of worthy metabolic compounds for industry and many of drug which used in treating human diseases (Kowalczyk *et al*., 2020).

MATERIALS AND METHODS

Sterilization of Seeds and planting

Seeds of *A. lebbeck* was washed from the soil stuck to them and were sterilized according to the method used before (Kdhim *et al.,* 2020). And Murashige and Skoog medium was prepared to cultivate sterilized seeds to obtain seedling according to the method (Hassan and Jassim,2018) after which the explant cotyledon was taken from them and cultivated on medium with different concentrations from 2, 4-D (0,1,2,3,4) mg / L and (0, 0.75,1.5) mg / L of BA for callus stimulation.

Increased and appreciated of Active substances.

The objective of increasing the amount of the four Budmunchiamine compounds (Fig. 1) studied in Albizia callus and increasing them, a constant weight 200 mg of callus was taken and implanted on The best combination of 2,4-D and BA (2, 0.75) mg/L respectively (Fig. 2) which gave highest weight of Callus with addition different levels of L-Arginine (0, 80, 160, 240, 320) mg /L. and callus brood at 24-26 °C at dark condition, after four weeks all callus were measurements were taken of the fresh and dry weight , in addition to 100 mg of callus was taken for each concentration of Arginine, while 2.5 grams of Albizia leaves were taken from plant *in vivo* for the purpose of assessing it with High-performance liquid chromatography (HPLC) device. Alkaliods identification with estimate Budmunchiamine through law Concentration of sample = Area of the sample/Area of the standard × Standard Conc. × Dilution factor According to the method described by Esfahani *et al*. (2008).

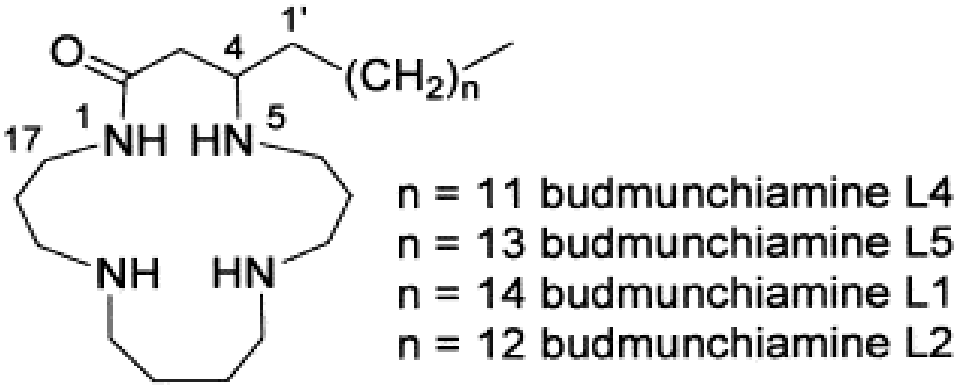


Fig. 1: Structure of budmunchiamine (Ovenden *et al*., 2002)

Fig. 2: The best callus of *A. lebbeck*

Statistical analysis

Completely randomize design for All experiments and to ten replicates follow program of SAS (2012) and to compare differences between treatments accorded to Least Significant Difference (LSD) ander 5% probability.

RESULTS AND DISCUSSION

The results of Table (1) showed that adding of 2, 4-D to the nutrient medium led to a significant increase in the weight of callus induced from cotyledon of the Albizia seedlings. One milligram per liter of 2, 4 – D was the most effective in this characteristic, and it was recorded 359.67, 23.37. mg, respectively for both of fresh and dry weight, compared to auxin-free treatment, the weight of the callus increases when the auxin concentration increases to reach the ideal concentration of 1 mg / liter of 2,4-D, then the decrease in callus induction begins with the increase in concentration. This may be due to the fact that high concentrations affect the work of the enzymes responsible for building the cell wall and its degradation, which affects the mechanical properties of the cell wall and affects cell division and callus formation (Taiz and Zeiger, 2002). However, the addition of BA to the medium led to significant differences in the dry and fresh weight of callus, as the BA concentration of 0.75 mg/L gave the highest fresh and dry weight of callus which reached 244.42 and 15.87 mg, respectively, compared to the neutral treatment. This may be due to the fact that cytokinins leads to cell division through rising transformation of the G2 phase to mitosis phase, because it raises the enzyme required by the mitosis phase. (Lee *et al*. 2011) have important effects as they increase the division of meristematic cells leading to an increase in the volume of different tissues of plant organs (Delloloio, 2007) The decrease in callus weighing induced from cotyledon with increase in the concentration of cytokines may be due to the attainment of the concentration of growth regulators (endogenous and additive to growth

medium) of inhibition levels of cell division and expansion (Mohammad and Yousif, 1982). The same table indicates that influence interaction between 2,4-D and BA at 2 mg / L and and 0.75 mg / L respectively was recorded highest rate the fresh and dry weight of Callus was 490.00 and 31.84 mg, respectively, and it was significantly different from most other treatments. Because Suitable explants to dedifferentiated state and begin to divide rapidly (Mutasher and Attiya 2019).

Table 1: Impact of 2, 4- D and BA in Fresh weight and Dry weight of callus induced from cotyledon.

2,4-D (mg/L)	BA (mg/L)						Mean fresh weight. (mg)	Mean Dry weight (mg)
	0		0.75		1.5			
	Fresh w.	Dry w.	Fresh w.	Dry w.	Fresh w.	Dry w.		
0	0.00	0.00	106.10	6.89	186.10	12.09	97.40	6.33
1	466.60	30.32	322.90	20.98	289.50	18.81	359.67	23.37
2	310.00	20.14	490.00	31.84	173.60	11.28	324.53	21.09
3	152.90	9.92	141.90	9.22	160.40	10.42	151.73	9.86
4	131.10	8.52	161.20	10.42	111.80	7.26	134.70	8.73
Mean	212.12	13.78	244.42	15.87	184.28	11.98	---	---
L.S.D.	2,4- D = 62.64 * BA= 48.52 * 2,4 D x BA= 108.49* Fresh weight							
* (P<0.05)	2,4- D = 4.07 * BA = 3.15* 2,4 D x BA= 7.05 * Dry weight							

Effect of L-Arginine on callus growth of *A. lebbeck*.

Table (2) shows that significant decrease in callus weighing with the gradual increase in the levels of Arginine to the MS medium, as the comparison treatment that was higher Fresh and dry weight reached 508.00 and 33.02 mg respectively, whilst treatment 320 mg/L of Arginine recorded the lowest Fresh and dry weight was 318.00 and 20.67 mg respectively.

Table 2: Effect of L- Arginine on callus weight of *A. lebbeck* after four weeks.

Conc. of L- Arginine (mg/L)	Fresh weight (mg)	Dry weight (mg)
0	508.00	33.02
80	426.00	27.68
160	388.00	25.22
240	364.00	23.60
320	318.00	20.67
LSD 0.05	19.34 *	1.25 *

The reason may be attributed to the fact that the addition of this precursor compound Arginine led to an increase in the osmotic pressure of the medium leading to a great effort in the culture medium, which works on the lack of water absorbed by the Cells, which leads to a reduction in preparedness to nutrients dissolved in the medium of cells than negative affects to the growth and development of callus cells (Yao, 2003). This direction in the decrease of fresh and dry weight in Table (2) was consistent with what Jassim found in (2018), which indicated a reduction in mean of fresh and dry weight to *Gardenia jasminoides* callus .and also in agreement with Kadhim and Jassim in (2020) that adding different concentrations of L-cystine to *Coriandrum sativum* medium with praise of the precursor added to MS medium it led to a gradual decrease in the rate of callus weight.

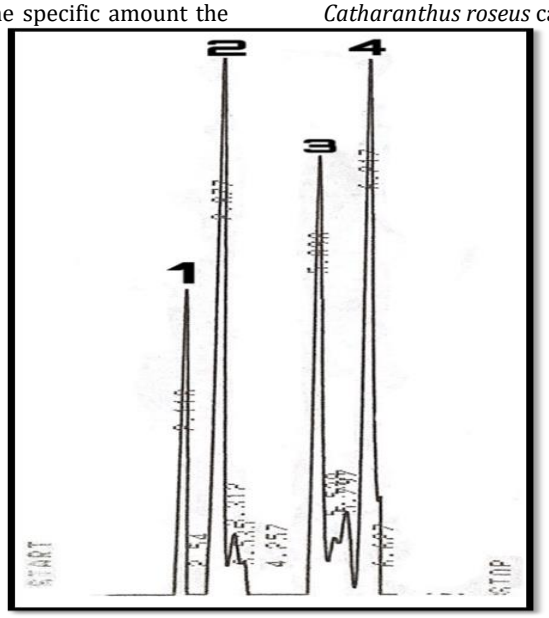
Effect of L-Arginine in the production of alkaloid compounds from callus.

Noted it from the results of Table (3) and Figures (3-8) that there are significant differences in the concentration of alkaloid compounds when different concentrations of L-Arginine are supplement in the MS medium , 320 mg/L which superiority in amount of Budmunchiamine L5 , Budmunchiamine L4, Budmunchiamine L2, Budmunchiamine L1 it reached 65.78 , 57.75, 44.79, 79.16 $\mu g.ml^{-1}$ respectively of fresh callus, while the comparison treatment gave the lowest value of Budmunchiamine L5, Budmunchiamine L4, Budmunchiamine L2, Budmunchiamine L1 which were 29.76 , 29.44 , 25.82, 23.72 $\mu g.ml^{-1}$ respectively.

Table 3. Effect of L-Arginine to enhance the alkaloid in *A. lebbeck* callus after five weeks of cultivation.

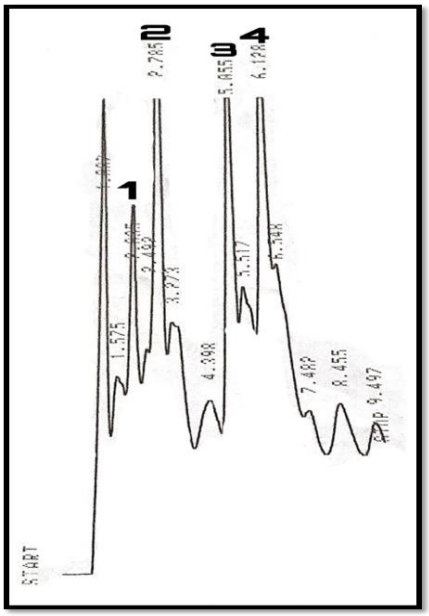
Conc. of Arginine (mg/L)	Budmunchiamine L5	Budmunchiamine L4	Budmunchiamine L2	Budmunchiamine L1
0	29.76	29.44	25.82	23.72
80	27.84	37.64	31.09	31.71
160	39.82	44.47	29.52	33.87
240	45.47	44.68	41.79	39.54
320	65.78	57.75	44.79	79.16
LSD 0.05	7.82 *	6.02 *	6.31 *	8.40*

The reason for this may be attributed to it being the synthetic precursor of the studied alkaloids the initiator is molecules directly Participates in the biosynthesis of the active compounds (Ramawat, 2008) and stimulates its produce often through rising the specific amount the artificial precursor or activating biosynthesis enzymes, or together (Demain, 1998). This is consistent with the results of Taha *et al*. in 2009 and what he found Jassim and Ameen in 2014. When adding the precursor to *Catharanthus roseus* callus.



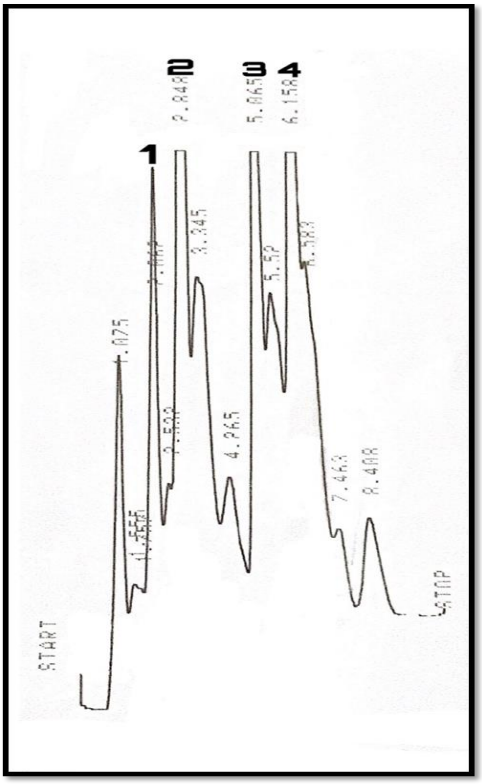
Abbreviations: 1 = Budmunchiamine L5, 2 = Budmunchiamine L4, 3 = Budmunchiamine L2, 4 = Budmunchiamine L1

Fig. 3. Standards curve of alkaloid compounds.



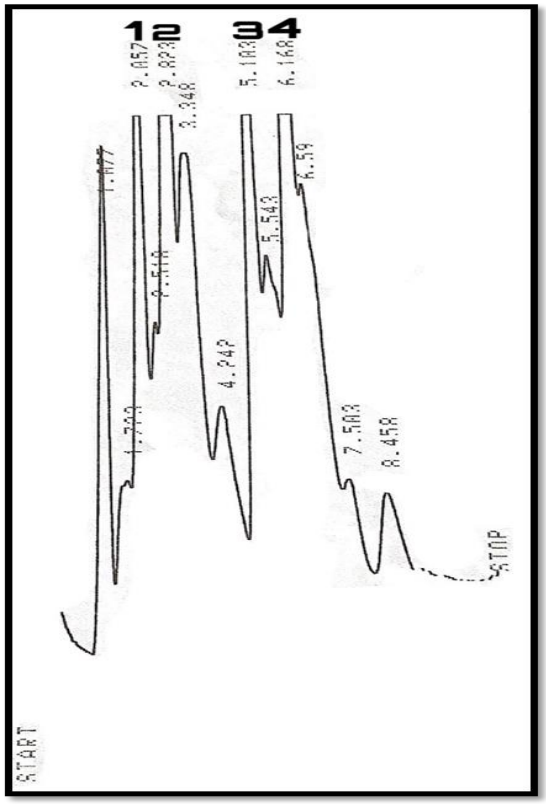
1 = Budmunchiamine L5, 2 = Budmunchiamine L4, 3 = Budmunchiamine L2, 4 = Budmunchiamine L1

Fig. 4. Alkaloid compounds in callus without L-Arginine.



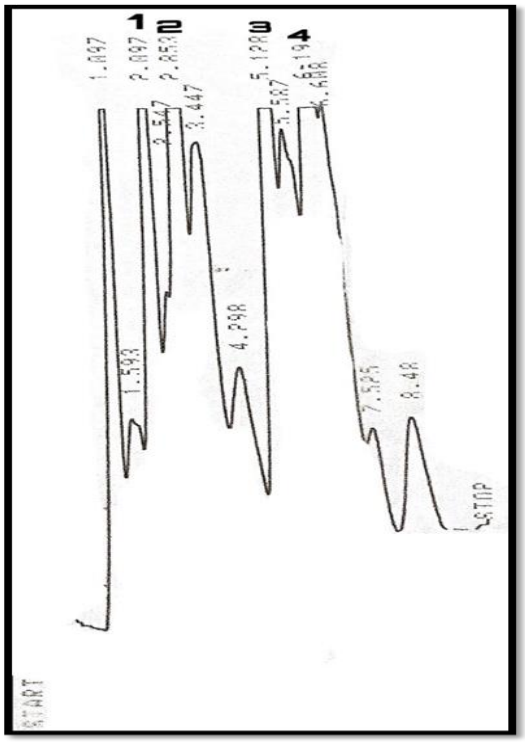
1 = Budmunchiamine L5, 2 = Budmunchiamine L4, 3 = Budmunchiamine L2, 4 = Budmunchiamine L1

Fig. 5. Alkaloid compound in callus at concentration (80 mg/L) of L-Arginine

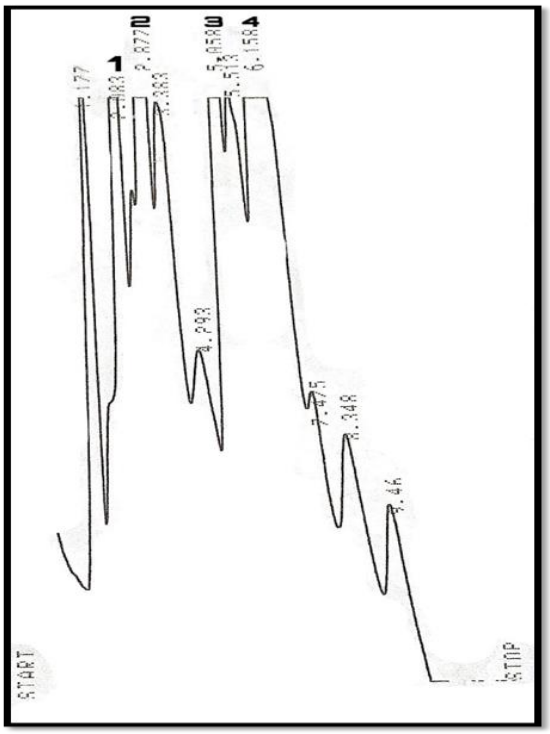


1= Budmunchiamine L5, 2 = Budmunchiamine L4, 3 = Budmunchiamine L2, 4 = Budmunchiamine L1

Fig. 6. Alkaloid compound in callus at concentration (160 mg/L) of L-Arginine.



1 = Budmunchiamine L5, 2 = Budmunchiamine L4, 3 = Budmunchiamine L2, 4 = Budmunchiamine L1
Fig. 7. Alkaloid compound in callus at concentration (240 mg/L) of L-Arginine.



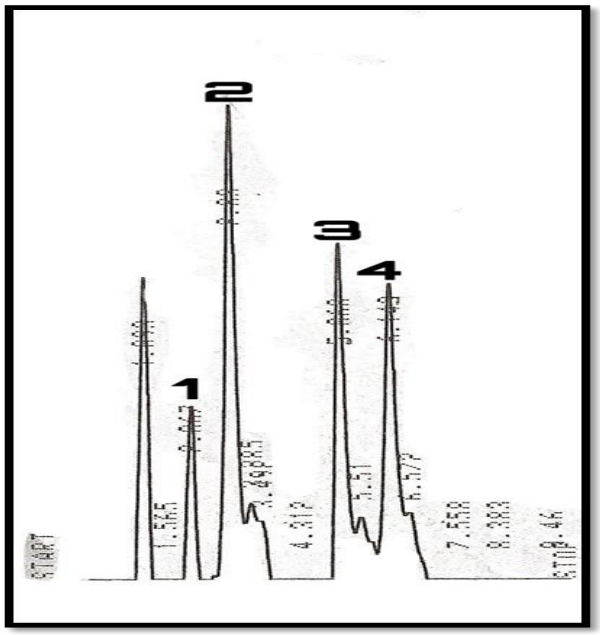
1 = Budmunchiamine L5, 2 = Budmunchiamine L4, 3 = Budmunchiamine L2, 4 = Budmunchiamine L1
Fig. 8. Alkaloid compound in callus at concentration (320 mg/L) of L-Arginine.

Assessment of secondary compounds in the leaves of *A. lebbeck*

Table (4) shows the types and quantities of active substances in plant leaves, as the HPLC device did not record the studied active substances when weights less than 2 grams of fresh cotyledon leaves of Albizia (Figure 9) also shows the number of active substances studied at a weight of 2000 mg of cotyledon leaves when compared with Those obtained from 100 mg of callus free-Arginine comparison shows the superiority of callus over the Albizia plant (*in vivo*). The reason may be that natural plants produce active substances in small quantities, which may reach (0.0005%) (Ebrahimzadeh *et al*., 1996).

Table 4. The Al kaloids (µg) in the cotyledon leaves extract and callus.

Al kaloids ($\mu g.ml^{-1}$)	Per 2000 mg from Cotyledon Leaf	per100 mg from callus
Budmunchiamine L5	11.39	29.76
Budmunchiamine L4	15.86	29.44
Budmunchiamine L2	10.86	25.82
Budmunchiamine L1	9.45	23.72



1 = Budmunchiamine L5, 2 = Budmunchiamine L4, 3 = Budmunchiamine L2, 4 = Budmunchiamine L1

Fig. 9. Alkaloid compound in cotyledon leaves extract.

CONCLUSION

Existence of L-Arginine in callus media with different levels are responsible for increasing of all compound of Budmunchiamine compare with Callus free L-Arginine which contain active substance more than the plant *in vivo*.

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