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CULTURE OF LAGOXILUS PLANT IN LABORATORY

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ABSTRACT

This paper provides information on the effects of Gibberellin A_3 (GA_3) and Auxin-indolyl-3-acetic acid (heteroauxin) stimulants on seed germination and growth for the culture of Lagochilus inebrians and the determination of lagoxilin diterpenoid by a known method in the literature.

KEYWORDS: *Lagochilus Inebrians, Lagochilus Pubescens, Gibberellin A_3, Auxin, Heteroauxin, Stimulator Infrared Spectroscopy, Lagoxillin, Thin Layer Chromatography.*

INTRODUCTION

Nowadays, the cultivation of promising plant species, the extraction of substances with high biological activity and the creation of new drugs based on them are developing rapidly around the world. Natural compounds extracted from plants have a high biological activity and have a special place in medical practice and in the national economy. Lagochilus inebrians Bunge is an intoxicating herb that is used as a sedative antihypertensive and anti-allergic agent. The main effect ingredient of the Lagoxilus plant is lagoxilin diterpenoid, a quaternary alcohol. Therefore, most lagochilus plants have hemostatic properties.

Main part:

Lagochilus is a perennial herb growing to a height of 20-60 cm. The stems are branched, ascending, the base is woody, four-sided, covered with hard glandular hairs. The leaves are simple, cut into three or five segments, opposite the band on the stem and branches. The flowers are pink, arranged in a semicircle on the stems and branches. The fruit is 4 nuts (fig. 1) and blooms in june-september. Lagochilus is harvested in july-august [1-5].

Fig. 1. Lagochilus inebrians Bge plant and its flower

The lagochilus plant grows in rivers, streams and rocks in the village of Navandak, Mirdosh, Langar of Akmal Ikramov collective farm of Xatirchi district, Nurata district of Navoi region of Uzbekistan. It is also found in Bukhara and Kashkadarya regions. It is grown in the village of Darmana on the former Frunze state farm in the Chimkent region. It is grown wild in the villages of Kushrabat, Gujumsay, Bazarjay, Jush, in Samarkand region. Lagochilus is named after the appearance of a rabbit's lip (from the Greek "lagos" - rabbit, "cheilos" - lip). Plants belonging to the genus Lagochilus belong to the family of lilacs (Lamiaceae or Labiamae).

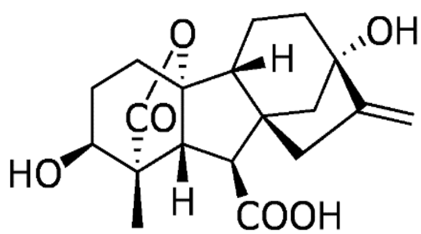
The chemical composition of Lagoxilus plant contains vitamin K1, 0,6-1,97 % lagoxilin, 0,67% flavonoid glycosides, 44-77 mg % ascorbic acid, 6-7 % organic acids, 5-10 mg % carotene, 9,66 -12,42 % resin, 2,58-2,78 % additives and other substances and also calcium and iron salts. Lagoxilus leaves contain lagoxilin, 0,03% essential oil, 11-14% additives, organic acids, 7-10 mg% carotene and 77-100 mg% vitamin C. The pharmacology of lagoxilus plant species has been studied in the pharmacology departments of Kuban, Samarkand and Andijan medical universities. In addition, aqueous and alcoholic decoctions of the species Lagochilus inebrians have been found to have hemostatic properties, physiologically active properties such as sedative, hypotensive, sedative, anti-shock, anti-radiation and desallergic (anti-allergic) [5-10]. As the demand for Lagoxilus-based preparations increased from year to year, the natural reserves of the wild-growing Lagochilus plant declined dramatically and are now listed in the Red book. To date, almost no practical work has been done on the cultivation of this medicinal plant in the country and the creation of cultivated plantations. Therefore, to study the effect of gibberellin A_3 (GA_3) and auxin-indolyl-3-acetic acid (heteroauxin) stimulants on seed germination and growth for culturing Lagochilus inebrians under laboratory conditions.

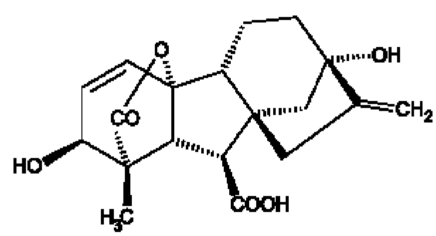
Gibberellins plant hormones regulate gibberellins plant growth and affect their developmental processes. These include stem elongation, germination, inactivity, flowering, enzyme induction, and leaf and fruit aging. Gibberellins were first discovered in 1926 by the japanese scientist Eiichi Khurasava [12].

It was first isolated from fungal species by Teijiro Yabutava Sumuki in 1935 and studied by Khurasava. Yabuta named the isolated substance as Gibberelin [12].

Interest in Gibberellins began outside Japan after world war II. The first scientific study of Gibberellin in the USA was conducted by Camp Detrick in Maryland. He tested gibberellin as a stimulant in a seedling of bean Vicia faba. The isolation of new species of gibberellins in the UK was initiated by large chemical industries. Interest in gibberellins has spread around the world, with its use in important beneficial plants becoming more prominent. For instance, research on this topic was conducted by David in California in the middle of 1960s. Thomson led the trial of gibberellins in seedless vines grown in special areas of California in 1962 [13].

Gibberellins are made with ent-gibberellan skeletons. When gibberellins were discovered, they were named GA_1 (Gibberellin A_1) according to the gan rule. Gibberellinic acid was the first Gibberellin GA_3 with a specific structure. In 2003, 126 Gibberellic acids were isolated from plants, fungi, and bacteria [12]. Gibberellins are tetracyclic diterpenic acids. At the base of each are 2 tattoos involving 19 or 20 carbons: 19-carbon gibberellins, for example, gibberellic acid does not contain 20-carbon, and instead has a five-membered lactone bridge bound to 4- and 10-carbon. 19-carbon states are, in general, biologically active forms of gibberellins. As well as highly effective in biologically active forms of hydroxylated gibberellins. In general, the most biologically active compounds are dihydroxylated gibberellins. Hydroxyl groups were involved in both the 3 and 13 carbons in them. Gibberellic acid is considered dihydroxylated [14].





Gibberellin A_1 (GA_1) **Gibberellic acid (GA_3)**

The Biological functions of gibberellins include various forms of cessation of stasis and stem elongation in the natural process. Before the photosynthetic apparatus is formed, it retains sufficient nutrient reserves of starch to feed the plant growing from seed in the early stages of stem elongation. Usually during stem elongation, the breakdown of starch into glucose begins in the endosperm after the seed is soaked in water. If plants are exposed to unfavorable temperatures, they produce large amounts of gibberellins. They enhance cell elongation, breakage and germination, seedless fruiting and seed germination. Gibberellins perform fully by stopping the inactivity of the seed and act as a chemical messenger. Its hormones bind to receptors, activate proteins, bind to complex DNA, and produce enzymes that enhance growth in the embryo. The main effect of gibberellins is to reduce proteins, and then again not to participate in the interaction of phytohormones and gene control [15].

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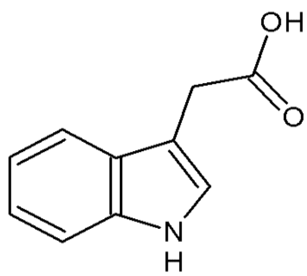
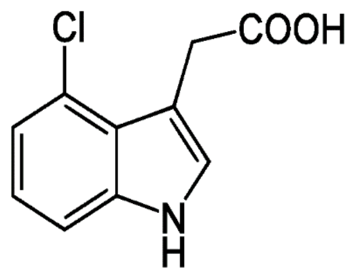
Auxins are a class of plant hormones (or plant-growing substances) that are distinguished by some morphologically similar properties. Auxins often play a key role in the normal growth of plant life processes. It also plays an important role in the development of the plant body. Auxins and their role in plant development were first demonstrated by dutch scientist Fritz Vent. Kennes Siman isolated this phytohormone and determined that its chemical structure was Indole-3-acetic acid [16]. Vent and Siman later became the author of the book phytohormones, or plant hormones, in 1937.

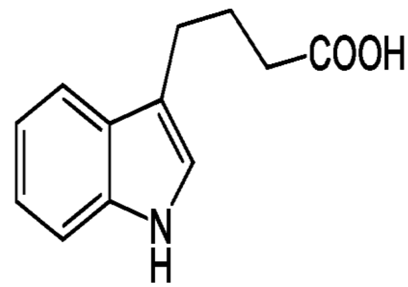
Local auxins indole-3-acetic acid is the most abundant local auxin found in plants and plays the most important role. It causes most of the effects of auxin in plants and is the strongest local auxin. There are more than three local endogenous auxins. All auxins are compounds with one carboxylic acid group bound by an aromatic ring.



Indol-3-acetic acid



4-chlorindol-3-acetic acid



Indole-3-butanoic acid

The name auxins is a greek word (auxein - growth). They were the first major plant hormones to be discovered. The distribution of auxin sample within the plant is the key factor for plant growth, the reaction of which is important for the environment and the development of plant organs. It transports molecules from cell to cell throughout the plant body through the active transport of a very complex and well-coordinated auxin (called polar auxin transport) [16]. Thus, the plant affects their appearance and regulates them without the need for a nervous system.

The specificity of auxins is that they interact with or counteract other plant hormones. For example, the ratio of auxin to sitochinin is determined by counting the buds in front of the root in plant tissue. At the molecular level, all auxins are compounds of the same carboxylic acid group bound by a benzene ring. The most important member of the auxin family is indole-3-acetic acid [13]. It is the strongest local auxin. Its stability as a local auxin has been tested in many ways in plants. For example, it has always been found that, under conditions, it generalizes the results obtained by reducing its molecules.

However, the molecules of indole-3-acetic acid are chemically variable in aqueous solution, so it is not used as a plant growth regulator. There are four natural (endogenous) auxins: indole-3-acetic acid, 4-chlorindol-3-acetic acid, phenyl acetic acid, and indole-3-butanoic acid. All of which have been identified separately from plants [16]. In addition to endogenous (local) auxins, scientists and manufacturers have developed many synthetic auxin compounds with auxinic activity.

Synthetic auxin analogues include 1-naphthalene acetic acid, 2,4-dichlorophenoxyacetic acid, and others. Some synthetic auxins, such as 2,4-dichlorophenoxyacetic acid and 2,4,5-trichlorophenoxyacetic acid, have been used as herbicides. As well as the sodium salt of 2,4-dichlorophenoxyacetic acid, a herbicide used to control weeds.

Broad-leaved plants (dicotyledonous), for example, rhubarb (dicotyledons), thin-leaved plants (single-leaved), for example, are more susceptible to auxins than grasses and cereals plants. Therefore, synthetic auxins are also useful as herbicides [12-16].

RESULTS AND DISCUSSIONS:

For the study of the effect of stimulants Gibberellin A_3 (GA_3) and Auxin – indolil-3-acetic acid (*heterooxine*) on the germination and growth of seeds, for the cultivation of Lagoche in inebrians plant, Gibberellin A_3 (GA_3) were extracted five different masses from gibberellin A_3 (GA_3) to prepare a solution of 10^{-4} M, 10^{-5} M, 10^{-6} M, 10^{-7} M and 10^{-8} m with a melted and dissolved in 2 liters of water. To prepare a solution of Gibberellin A_3 with 10^{-4} M li, Gibberelin A_3 (Mr=346,4 gr/ mol) was taken from 0,06928 gr or 69,28 mg, and for 10-5 M li was taken from 6,928 mg and dissolved in 2,0 liters of water. Then it was postponed for a day. The remaining solutions are prepared in the same way. For the preparation of solutions of auxin – indolil-3-acetic acid (*heterooxine*) 10^{-4} M, 10^{-5} M, 10^{-6} M, 10^{-7} M and 10^{-8} m, five different masses of auxin was taken and dissolved in 2 liters of water. From Auxin (Mr=175,184 gr/mol) were taken 0,350368 gr or 350,368 mg for the preparation of 10^{-4} M li solution of Auxin and also for 10^{-5} M was taken 35,0368 mg and dissolved in 2 liters of water. Then it was postponed for a day. The remaining solutions are prepared in the same way, and the seeds of the plant 10 units are soaked for 24 hours in the above solutions, and the soil:sand:manure prepared in the proportions of 2:1:1in the pans, planted at a depth of 3-5 cm. The main indicators of the plant grown under the influence of solutions of Gibberillin A_3 and Auxin – indolil-3-acetic acid (*heterooxine*) 10^{-4}, 10^{-5}, 10^{-6}, 10^{-7}, 10^{-8} M are presented in Table 1.

TABLE 1 EFFECT OF GIBBERILLIN A_3 AND AUKSIN-INDOLIL-3-ACETIC ACID (HETEROAUKSIN) ON THE GERMINATION AND GROWTH PROCESS OF LAGOXILUS PLANT SEEDS

The drug concentration, mol	Time of germination of seed (day)	The length of the plant for 8 days (cm)	Number of plants grown from 10 seeds	The degree of germination of seeds (%)	Length of the root of the plant, cm
Control GKMAT	9-12	3,8	3	30	12,6
Gibberellin A_3					
10^{-4}	7	5.2	4	45	13,5
10^{-5}	6	5.9	5	60	13,6
10^{-6}	7	5.7	4	50	13,4
10^{-7}	8	5.3	4	45	13,3
10^{-8}	10	4.8	4	55	13,2
Auxin-indolil-3-acetic acid					
10^{-4}	7	5,2	4	45	14,6
10^{-5}	6	5,6	4	50	15,0

10^{-6}	7	5,8	5	55	15,5
10^{-7}	8	5,1	4	60	14,8
10^{-8}	10	4,9	2	50	14,3

From the indicators presented in Table 1, it can be seen that the growth rate of the lagoxilus plant and the degree of fertility of its seeds, the time of germination, the length of the plant and its root is 10^{-4}-10^{-5} M of gibberellin A_3 and auxins, the length of the plant and leaves under the influence of concentrations it was found to have an effective effect on the increase in the number of seeds and 10^{-4},10^{-5} concentrations of plant seeds increased from 40% to 60%. The length of the plant was 5,6 cm, the length of the root was 14,3 cm. The seeds of plants germinated on 7-10 day. The most optimal for the growth process of the plant is the use of solutions of this concentration when planting the lagoxilus plant from seeds in field conditions. In control, the seeds of the lagoxilus plant sprouted between 9-12 days, the length of the plant was 3,8 cm, the length of the root 12,6 cm. The degree of fertility of plant seeds was observed to be from 20% to 30% in control.

In the experimental section, Gibberellin A_3 (GA_3) was taken from Gibberellin A_3 (GA_3) to prepare the solutions and dissolved in 2 liters of water. To prepare a solution of Gibberellin A_3 with 10^{-4} M li, Gibberelin A_3 (Mr=346,4 gr/ mol) was taken from 0,06928 gr or 69,28 mg, and for 10^{-5} M li was taken from 6,928 mg and dissolved in 2,0 liters of water. Then it was postponed for a day. The remaining solutions were prepared in the same way. For the preparation of solutions of Auxin – indolil-3-acetic acid (heterooxine), the mass of five different was taken from the Auxin and dissolved in 2 liters of water. From Auxin (Mr=175,184 gr/mol) were taken 0,350368 gr or 350,368 mg for the preparation of 10^{-4} M li solution of Auxin and also for 10^{-5} M was taken 35,0368 mg and dissolved in 2 liters of water. Then it was postponed for a day. The remaining solutions are prepared in the same way.

For the preparation and processing of soil, ordinary soil, sand and manure were obtained. Soil: sand: manure ratio is obtained from 2:1:1 (by mass) and mixed well with each other. Then put in special containers, prepared for planting. The small stones were placed with 3,0-3,5 cm before covering the mixture of soil:sand :manure on the bottom of special dishes. The goal of it is to grow the sprouted plant well and let the air enter from the bottom of the container. The ground was made ready for planting.

For processing Lagoxilus seeds, Lagoxilus plant seeds were taken and treated with each solution. This process was carried out as follows. The lagoxilus seeds were soaked in different concentrated solutions of each substance being tested, and a day later sown at a depth of 1,5-2,0 cm, corresponding to each container.

Figure 2. Growth process of Lagochilus inebrians Bge plant

The seeds were watered after planting. Each process was followed. Room temperature and light were given the same.

CONCLUSION

Growth and development of the Lagoxilus plant at gibberellin A_3 and 10^{-5} and 10^{-6} molar concentrations of auxin were found to increase the root and stem length of the lagoxilus plant. The formation and proliferation of lateral and additional roots under the influence of auxin.

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