PARASITIC PLANTS AND THEIR PHYSIOLOGICAL INTERACTIONS IN THE NATURAL ECOSYSTEMS

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ABSTRACT

This paper presents the results of a research which had been carried out on the physiology of the parasitic plants *Cuscuta campestris* Yunker and *Orobanche caryophyllacea* Smith.

The physiological indices which have been analyzed were: respiration, transpiration, total water content, bound and unbound water, osmotic pressure, suction force, the phosphorus and potassium contents.

The recordings show an increased value for the transpiration, a high content of total water, a low percentage of bound water and high values of osmotic pressure and suction force. The data obtained after these studies had been compared with the recordings from the host plants.

The results from the host plants indicate the following: reduced osmotic pressure of the cellular juice, a reduced suction force and a lower overall water content.

The ash content is greater in the case of the parasite plants, compared with their hosts.

At the same time, the phosphorus and potassium contents are clearly superior when compared with the values determined for the host plants.

INTRODUCTION

Parasitic nutrition is a mode of heterotrophic nutrition where an organism lives on the body surface or inside the body of another type of organism.The parasite obtains nutrition directly from the body of the host. The parasites derive their nourishment from their host. This symbiotic interaction is often described as harmful to the host. Parasites are dependent on their host for survival; host provides nutrition and protection for the parasite. As a result of this dependence, parasites have considerable modifications to optimize parasitic nutrition and therefore their survival. (https://parasitology.conferenceseries.com/events-list/nutrition-and-biochemistry-of-parasites).

Even tough, until recently, the parasite plants have been regarded as harmful, this perception has drastically changed at present. Many of them are actually protected at European or global level, others have a recognized therapeutic value due to the compounds contained in their tissues, being useful in treating different kinds of affections.

Parasites require nutrients to carry out essential functions including reproduction and their growth. The nutrients required from the host are, amino acids, carbohydrates and lipids. Carbohydrates are utilised to generate energy, amino acids and fatty acids are involved in the synthesis of macromolecules and the production of eggs. Most parasites are heterotrophs, so they are unable to synthesise their own 'food' (*https://parasitology.conferenceseries.com/events-list/nutrition-and-biochemistry-of-parasites).*

Parasitic plant, is a plant that obtains all or part of its nutrition from another plant (the host) without contributing to the benefit of the host and, in some cases, causing extreme damage to the host. The defining structural feature of a parasitic plant is the haustorium, a specialized organ that penetrates the host and forms a vascular union between the plants. (https://www.britannica.com/plant/parasitic-plant)

Parasitic plants can be categorized based on different criteria such as where they attach to the host, the degree of nutritional dependence upon the host, or whether they require a host to complete their life cycle. In terms of location on the host, two basic types can be distinguished: stem parasites and root parasites. Stem parasites occur in several families, and pathogenic members include some mistletoes and dodder (*Cuscuta* and *Cassytha*). Root parasites are more common and occur in diverse taxonomic groups. Some of the most economically important root pathogens are in the broomrape family, *Orobanchaceae.*(Nickrent,D.L,Musselman L.J. 2004).

Dodders may be the most important parasitic weeds of legumes in temperate regions. Of particular importance is *C. campestris* on alfalfa (*Medicago sativa*) with especially significant impact on alfalfa grown for seed. The alfalfa and dodder seeds are similar in size, and so the parasite is spread with the host. The wide range of hosts attacked by dodders is reviewed in Dawson et al. (1994). The most effective means of control is seed sanitation. Because the surface of dodder seeds is minutely roughened, dodder seeds stick to felt rollers while alfalfa seeds pass over. Dawson et al. (1994) also reviewed several herbicide treatments that are directed at the newly germinated seeds of dodder. (Nickrent, D.L., Musselman, L.J. 2004)

Dodder seeds can be stored for several years in the soil. After germinating on the surface or emerging from the soil, plantlets without roots emerge which will gradually cover the tomato plant through twining stems, and parasitize the stems and leaves through suckers or haustoria. These provide access to the vascular system of the plant and so remove the minerals, metabolites, and water essential for their development, without recourse to photosynthetic activity. Infected tomato plants have a decreased growth rate, especially if they are parasitized early . In the latter case, they can even die. https://www.sciencedirect.com/topics/agricultural-and-biological-sciences/parasitic-plants

MATERIALS AND METHODS

For the study of the physiological processes and of some parameters, determinations were made on the species: *Cuscuta campestris (fig. 1) and Orobanche caryophyllaceae(fig 2)*

The genus *Cuscuta* from *Convolvulaceae* family is an obligate stem parasite (Parker and Riches, 1993; Dawson et al., 1994;).

The genus *Cuscuta* has widely been placed in its own family *Cuscutaceae,* but most authorities (e.g. Flora Europaea, Missouri Botanic Gardens) now merge *Cuscutaceae* into *Convolvulaceae.* Pérez-Amador et al. (1996) considered this was supported by evidence of taxonomic markers, typical of Convolvulaceae, also occurring in *Cuscuta* spp., and Stefanovic and Olmstead (2004) present further evidence from phylogenetic studies.

Cuscuta campestris is the most widespread species in the genus in the world and the only parasitic weed of North America that has spread to the Old World (Dawson et al., 1994). It obtains its

resources entirely from its host plants, severely suppressing
them and even resulting in their death (Ashton and Santana, 1976; Cooke and Black, 1987; Dawson et al., 1994).

This parasite has a wide range of host species (Parker et al., 1984). It mainly parasitizes alfalfa, but also attacks some horticultural crops, legumes, and broadleaved weeds, though it is seldom found on woody plants, grasses, or cereals (Dawson et al., 1994).

Although it normally grows as an annual (Dawson et al., 1994), its shoots can stay alive in winter, and its seeds may germinate and then infect host plants in the following spring (Wang et al., 2002,cited by Hao Shen, Wanhui Ye et al, 2005).

The parasitic weed *C. campestris* is native to North America, but has been introduced around the world and become a weed in many countries. It is by far the most important of the dodders, perhaps because of its wide host range. This ensures that there is a wide range of crop seeds that may be contaminated, and in which it may be introduced to new areas over both short and long distances. Once introduced it is almost certain that there will be suitable host plants on which it can thrive and be damaging, whether they are crops or wild species. Vegetative spread can be very rapid – up to 5 m in 2 months. It also has a wide tolerance of climatic conditions from warm temperate to sub-tropical and tropical. https://www.cabi.org/isc/datasheet/17111#tosummaryOfInvasiveness

Cuscuta species have a very distinct appearance, consisting mainly of leafless, glabrous, yellow or orange twining stems and tendrils, bearing inconspicuous scales in the place of leaves.

In *C. campestris*, the yellow to pale orange true stems, about 0.3 mm in diameter, generally do not twine and attach to the host, but produce tendrils of similar appearance, arising opposite the scale leaves, which do form coils and haustoria (Dawson, 1984).

The seedling has only a rudimentary root for anchorage, while the shoot circumnutates, i.e. swings round anti-clockwise about once per hour, until it makes contact with any stem or leaf, round which it will coil before growing on to make further contacts.

Fig. 1. *Cuscuta campesris- young stem*

The root and shoot below this initial attachment soon die, leaving no direct contact with the soil. Haustoria form on the inside of the coils and penetrate to the vascular bundles of susceptible hosts. Flowers, each about 2 mm across, occur in compact clusters 1-2 cm across. There is a calyx of 5 fused sepals with obtuse or somewhat acute lobes, and 5 corolla lobes, triangular, acute, often turned up at the end, equalling the length of the tube. Stamens alternate with the corolla lobes, each with a fringed scale below. The ovary is almost spherical with a pair of styles with globose tips. The capsule reaches 2-3 mm across when mature, with a depression between the two styles. The capsule does not dehisce and seeds remain on the plant long after maturity. Seeds are irregular in shape, rough-surfaced, about 1 mm across. https://www.cabi.org/isc/datasheet/17111#tosummaryOfInvasiveness

Orobanche caryophyllacea Smith, family *Orobanchaceae (fig 2).*

Fig.2. Orobanche cariophylacea

Herbs 35-50 cm tall. Stems 15-30 cm, sparsely glandular pubescent upward. Leaves ovate-lanceolate or lanceolate, 2-2.5 cm X 4-6 mm, glabrous adaxially, glandular pubescent at margin and abaxially. Inflorescences spicate, 10-20 cm; bract ovate-lanceolate, 2-3 cm X 4-5 mm, along with calyx densely glandular pubescent; bractlets absent. Calyx 1-1.2 cm, irregularly 2-parted; segments lanceolate, entire or 2-lobed; lobes linear-lanceolate, unequal, 4-8 mm, usually 3-veined, apex long acuminate. Corolla yellow, 2-3.5 cm; tube not constricted, distinctly enlarged upward; upper lip slightly emarginate or mucronate; lobes subrounded, unequal, glandular pubescent at margin and on both sides, margin irregularly dentate or sinuolate. Filaments 1.2-1.4 cm, white villous proximally, glabrescent distally; anthers oblong, 1.8-2 mm, white pubescent. Pistil ca. 1.6 cm; ovary narrowly ellipsoid. Style ca. 1 cm, glandular pubescent; stigma 2-lobed, lobes globose, ca. 1 mm in diam. Capsule oblong, 1-1.2 cm. Seeds oblong, 0.4-0.5 X ca. 0.3 mm. Fl. May-Jul, fr. Jul-Sep. 2*n* = 38.Parasitic on species of *Galium* Linnaeus.
http://www.efloras.org/florataxon.aspx?flora_id=2&taxon_id=200021469

The experiments have been carried out in the Balutei's Keys, Mehedinti Plateau, Romania.

Placed in Mehedinti Plateau, Balutei Keys are the only keys of great size and importance from the territory of Ponoarele. They have a length of about 1 km and are located near the village of Baluta, being bordered by Cornetul Balutei and Raienilor, with limestone walls with heights measuring between 300-400 m. The mild climate, with sub-Mediterranean influences alongside with the geological structure and relief, were favorable conditions for the formation and preservation of a vegetation that has a great scientific value, as well as for a great landscape (Buse Dragomir Luminita et al, 2018)

The climate is continental temperate, with slight sub-Mediterranean influences.

The determinations have been conducted during May and September 2020 and consisted in a number of measurements of the physiological indices at two species of parasite plants: *Cuscuta campestris* and *Orobanche carophyllacea.*

In parallel, measurements have been conducted on the host plants. In the case of *Cuscuta,* the host plant was *Medicago minima* and in the case of *Orobanche*, the host plant was *Galium erectum.*

The analyzed physiological indices have been the respiration intensity, transpiration intensity, total water content, the water types (bound and unbound water), the concentration of the cellular juice, osmotic pressure, the content of pigments and the content of phosphorus and potassium.

Respiration and transpiration were determined with the portable Lci apparatus.

The total water content was determined gravimetrically by drying the plant material at the oven at 105 ^{0}C.

The water forms(bound and unbound) were determined by the Artihovski method (Boldor O., 1983).

The suction strength of the parenchyma was determined by immersing equal portions of haustoria in solutions with different concentrations of sucrose and determining the isotonic solution (Boldor O., 1983).

The concentration of the cellular juice has been determined with a refractometer from the juice obtained after pressing the vegetal material with a specially designed stainless steel press.

The osmotic pressure of the cell juice was determined using the plasmolytic method. (Boldor O., 1983).

The quantity of pigments from the leaves has been determined spectrophotometrically from the extract obtained from a gram of milled leaves and acetone 80% in a final volume of 50 ml of substance.

The extinction has been determined for a wavelength of 646, 663, 470 nm, and the quantity of pigments has been calculated with the following formulas:

Chlorophyll a (mg/100g) = (12,21 • D663) – (2,81 • D646) • 5

Chlorophyll b (mg/100g) = (20,13 • D646) – (5,03 • D663) • 5

Carotene + xanthophyll (mg/100g) = (1000 • D470) – (3,27 • cl a–1,04 cl b)

The ash has been obtained by incinerating the vegetal material at 550 degrees Celsius.

The phosphorus content (P_2O_5) has been determined spectrophotometrically. The process is based on the following property of the phosphoric acid: it gives a yellow color in contact with the ammonium molybdate.

The vegetal material is mineralized with the wet procedure, with the help of a mixture of perchloric acid, sulfuric acid and hydrogen peroxide.

A mixture of KH_2PO_4, ammonium molybdate and acetone is used as the standard solution (Buliga,E., Unc, R., 1996).

The potassium quantity has been determined through a volumetric procedure

This method consists on the precipitation of potassium from the hydrochloric ash solution in the form of sodium cobalt nitrite and potassium.

Subsequently, the cobalt nitrite is decomposed in an acidic environment with the excess of 0.1 n potassium permanganate. The excess of permanganate is titrated with oxalic acid (Buliga,E., Unc, R., 1996).

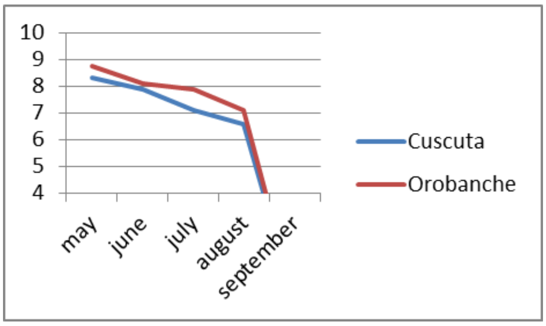
RESEARCH RESULTS

Transpiration intensity

The transpiration intensity shows high levels at both parasitic species. Higher values have been recorded for *Orobanche* in the same temperature and humidity conditions.

These values indicate an increased capacity to absorb with the help of suckers the elaborated sap from the tissues of the host plants.

These values are influenced by the environment conditions. As a result of this, during the spring period, the maximum registered value is 8,93 mmol / m ^{2} / s and, during the summer time, the maximum registered value is 7,46 mmol / m ^{2} / s.



Graph. 1. The seasonal variation of leaves transpiration (mmol / m ^{2} / s)

Respiration intensity

The respiration intensity is greatly influenced by the temperature: the highest values have been recorded at 32 degrees Celsius (the maximum temperature during the experiments): 9,6 (µmol /m^2 /s). The minimum value has been recorded at 10 degrees Celsius, during the spring time: 6,5 µmol /m ^{2} /s (graph.2).

Recordings being carried out at the same temperature (20 degrees Celsius), but during different life cycle periods show that the respiration is also dependent on the stage of development of the parasitic plants. During the flowering period, this value reaches its maximum.

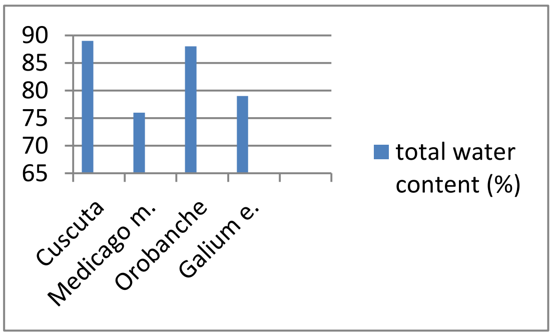
Graph. 2. The seasonal variation of respiration (μmol /m^2 /s)

The total water content

The total water content has been determined at both the host and parasite plants in June.

The registered values show an accumulation of water in the tissues of the parasitic plants, clearly superior when compared with the same determination made on the host plants (graph 3).

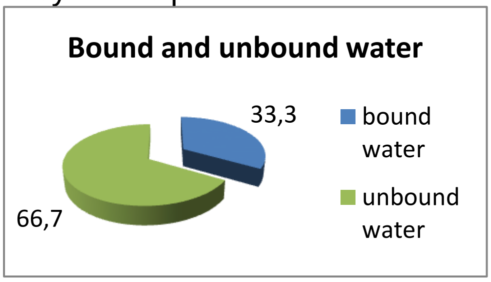
This can be a direct consequence of the increased suction force recorded for the parasitic plants, but also a result of the fact that the host plant, debilitated, shows a weak absorption of water from the soil level.



Graph. 3. The total water content of parasitic plants and host plants (%)

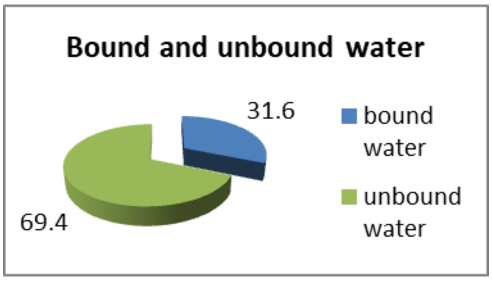
The bound and unbound water

The water types for the parasitic plants have been recorded during the same time period (month of June) and they indicated an increased percent of bound water when compared with the values that are normally recorded in photosynthetic plants.



Graph. 4. The content in bound and unbound water in *Cuscuta campestris* (%)

The highest values have been recorded in the case of *Cuscuta campestris* during the summer time (graph.4).

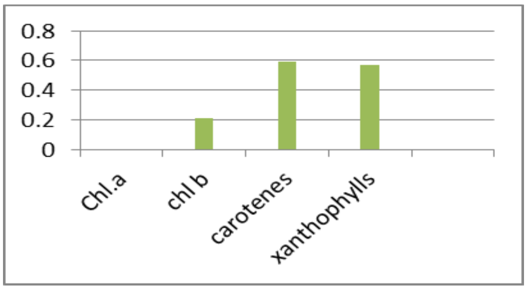


Graph. 5. The content in bound and unbound water in *Orobanche c.* (%)

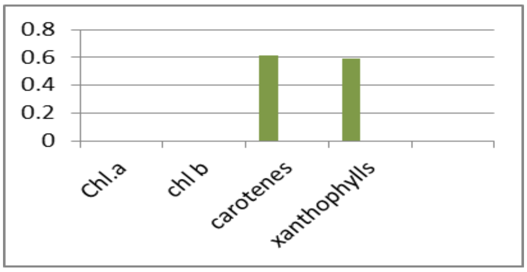
The pigments content

Spectrophotometric determinations of the quantity of pigments for the two parasitic species which have been studied indicated a predominance for the carotenoids (carotenes, xanthophylls) (graph. 6,7).

In the case of *Cuscuta*, even though it is a totally parasitic species, the stems, especially the young ones, also contain a quantity of **b** chlorophyll: 0,214 g/ 100 g veg matter but the **a** chlorophyll has not been identified (graph 6).



Graph. 6. The content in pigments in *Cuscuta campestris* (g/100g veg. mat)



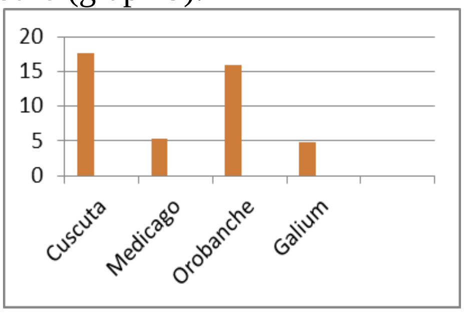
Graph. 7. The content in pigments in *Orobanche* (g/100g veg. mat)

The concentration of the cellular juice and the osmotic pressure

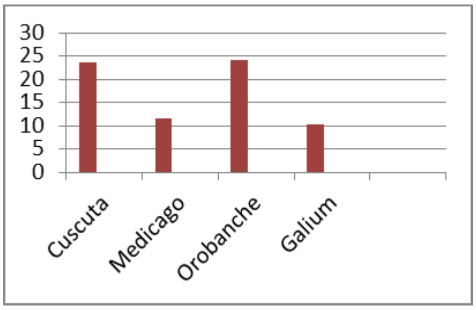
The concentration of the cellular juice, as well as the transpiration, depend not only on the behavior of the parasite, but also on the external conditions and, most importantly, on the water inflow at the tissue level.

The increased concentration of the cellular juice (graph. 8) is a direct consequence of the accumulation of sugars in the tissues of the parasitic plant.

The modification of the cellular juice concentration also leads to a change in the value of the osmotic pressure (graph 9).

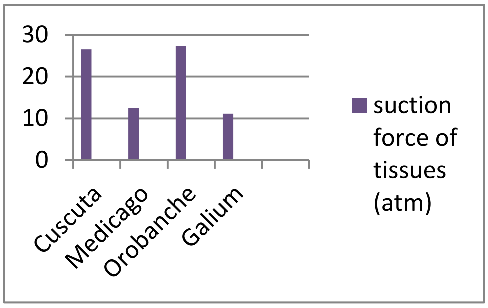


Graph. 8 The concentration of the cellular juice in parasitic plants and their hosts (% soluble dry matter)



Graph. 9. The osmotic pressure of the cellular juice (atm)

The suction force of tissues showed high values, of more than 20 atmospheres at both parasitic plants taken into the study. The maximum value of 27.3 atmospheres has been recorded at *Orobanche* during the flowering period which requires the largest quantity of energy (graph.10)

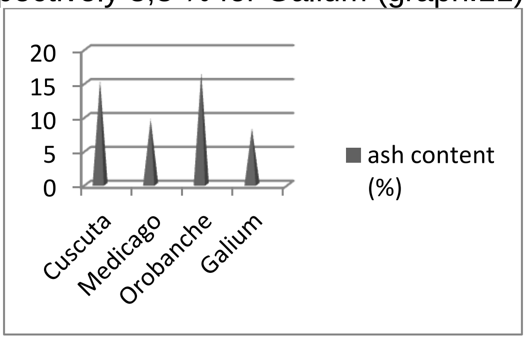


Graph. 10. The suction force of the tissues (atm)

The ash content

The parasitic plants, when burnt, give a larger amount of ash, compared with the host plants: *Cuscuta* 15,4%, *Orobanche* 16,6% *.*

In the host plants the ash percentages were: 9.7% for Medicago, respectively 8,3 % for Galium (graph.11).



Graph 11. The ash content of parasitic plants and their host plants (%)

The phosphorus content (P_2O_5)

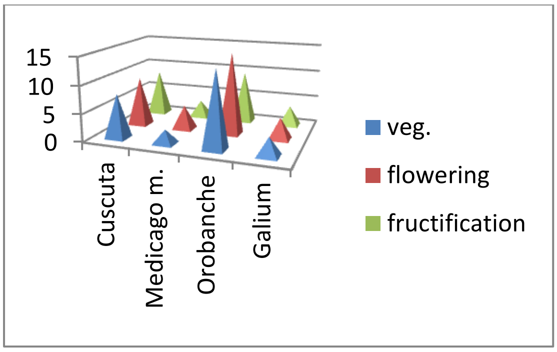
P is an important plant macronutrient, making up about 0.2% of a plant's dry weight. It is a component of key molecules such as nucleic acids, phospholipids, and ATP, and, consequently, plants cannot grow without a reliable supply of this nutrient. Pi is also involved in controlling key enzyme reactions and in the regulation of metabolic pathways (Theodorou and Plaxton, 1993). After N, P is the second most frequently limiting macronutrient for plant growth.

Under normal physiological conditions there is a requirement for energized transport of Pi across the plasma membrane from the soil to the plant because of the relatively high concentration of Pi in the cytoplasm and the negative membrane potential that is characteristic of plant cells. This energy requirement for Pi uptake is demonstrated by the effects of metabolic inhibitors, which rapidly reduce Pi uptake. (Schachtman, P., D.,et al, 1998)

The percentage of phosphorus in parasitic plants is clearly higher than the percentage of phosphorus in host plants

The concentration of phosphorus increases slightly until the flowering period (8,93% at Orobanche, and 7,54 at Cuscuta) and then gradually decreases when plant reaches maturity.

In the host plants, the maximum percentage of ash recorded in the flowering phase was 4.40 for *Medicago* and 3.31 for *Galium* (graph. 12).



Graph 12.The percentage composition of phosphorus (P_2O_5) in host and parasite plants (% of ash)

The potassium content

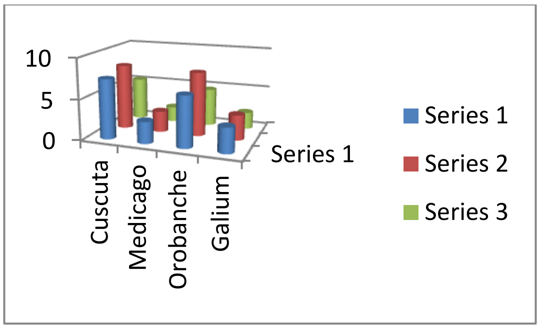
Potassium plays an important role in the normal activity of plants, facilitating numerous enzymatic reactions. The intervention of potassium, as well as phosphorus, in different stages of sugar formation and circulation is well known.

Tissue age is an important factor in the accumulation of potassium. In young plants, potassium accumulates in larger quantities compared to other periods in the life cycle (Singh, J.,N., Lal, K.N., 1961).

At the host plants, the potassium concentration is greater in the stems than in leaves. In the stems, the potassium percentage increase until the flowering period; afterwards it remains constant until the seeds have formed, when a slight decrease is recorded.

The parasitic plants contain a great quantity of potassium (graph13).

The large influx of potassium in this plants is probably determined by its participation in the circulation of sugars.



Graph. 13.The percentage composition of potassium (K_2O) in host and parasitic plants (% of ash)

CONCLUSIONS

- The parasitic species contain large amounts of carotenoid pigments from the carotene and xanthophyll group and small amounts of chlorophyll, only from the b group.
- The intensity of respiration in parasitic species shows very high values, the process being influenced by environmental factors, mainly the temperature
- The parasitic plants showed very high values in the case of the suction force, this force being necessary for the absorption of large amounts of elaborate sap
- The water content of parasitic plants is higher than the water content of their hosts
- The amount of ash is higher in parasitic plants than in host plants and its analysis shows that phosphorus and potassium are in much higher amounts in the tissues of parasitic plants. From this point of view, the ash composition of parasitic plants approaches the ash composition of embryonic or reserve tissues.

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