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RESEARCH REGARDING THE CORRELATION BETWEEN TOTAL LIPIDS CONTENT AND BIOSYNTHETIC CAPACITY AT DIFFERENT ALKALOID TYPES STRAINS OF *CLAVICEPS PURPUREA* SCLEROTIA

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INTRODUCTION

The interest in ergot alkaloids is great because theirs derivatives that are potentially therapeutic agents for parkinsonian, acromegaly, amenorrhea-galactorrhea, suppression of postpartum lactation, treatment of breast cancer and possibly cancer of the prostate, migraines and the symptoms associated with them, ortostatic circulatory perturbations, senile cerebral insufficiency, hypertension as well other affections in which their antibacterial, hipolipemic and immune-regulator effects are studied (DESAI, J.D. at al., 1983; SURDU et al., 2005).

The lipid metabolism is associated with alkaloid biosynthesis because the common precursor acetyl-CoA. The literature mentions that lipid accumulation, products of primary metabolism, parallels alkaloid yield in some cases (there is no evident competition for their synthesis) or, the amount of fatty acids increases until the beginning of alkaloid biosynthesis in other cases.

In the last case, after the release of alkaloids biosynthesis process the total lipids content is approximately constant. More, it is considered that the presence of lipids inclusion is typical of strains capable to produce alkaloids (SURDU S. et al.).

Some researches results substantiate that a common regulatory moment or moments participates in the synthesis of alkaloids and lipids. The main components of the lipids are linoleic acid, palmitic acid, oleic acid and in small amounts palmitoleic acid, stearic acid and ricinoleic acid.

Generally, the strains of *Claviceps purpurea* have different biosynthetic alkaloid capabilities and predominantly produce a certain peptide alkaloid.

MATERIALS AND METHODS

The biological material is represented by ergotamine and ergocristine type dry sclerotia harvested from Ergo race autumn rye plants artificially infected with conidia suspension of *Claviceps purpurea* obtained in submerged cultures. There have been used five strains of each alkaloid type.

For total alkaloid amount determination, the Rumpel method has been employed (RUMPEL, W., 1955). It consists of alkaloids extraction with a methanol solution of tartaric acid and extract purification with zinc acetate. The extract reacts with the sulphuric solution of p-dimethylaminobenzaldehyde, forming a blue compound, measurable by photoelectric colorimeter.

For total lipids determination we used the method described by Soxhlet, the most commonly used example of a semicontinuous method applied to extraction of lipids. According to the Soxhlet's procedure, oil and fat from solid material are extracted by repeated washing (percolation) with an organic solvent, usually hexane petroleum ether or ethyl ether under reflux in a special glassware (ARTENIE V., TĂNASE E., 1981).

The results reported here are average values from three independent determinations and have been statistically processing.

RESULTS AND DISCUSSIONS

The mature sclerotia, harvested from Ergo race autumn rye plants, was dried at 50^0C until constant weight. The strains utilised have been conventionally noted, after the predominant alkaloid, T1, T2, T3, T4, T5 and S1, S2, S3, S4, S5 for ergotamine (T) and respectively ergocristine (S) types.

The total alkaloid content from the first harvest of ergocristine and ergotamine sclerotia is presented in table I.

Table I. The total alkaloid content CAT) of ergosristine and ergotamine type *Claviceps purpurea* sclerotia

Crt. No.	Strain type	CAT(g%)
1	S1	0,64
2	S2	0,67
3	S3	0,69
4	S4	0,70
5	S5	0,72
6	T1	0,61

7	T2	0,61
8	T3	0,68
9	T4	0,75
10	T5	0,79

The data were statistically analysed for both alkaloid types (table II).

Table II. Statistical analysis of total alkaloid content of ergocristine and ergotamine type *Claviceps purpurea* sclerotia

Crt. No.	Statistical indicator	Ergocristine strains	Ergotamine strains
1	Mean	0,684	0,688
2	Standard error	0,013638182	
3	Median	0,69	
4	Mode	#N/A	#N/A
5	Standard deviation	0,030495901	0,081363382
6	Sample variance	0,00093	0,00662
7	Kurtosis	-0,003468609	-2,379450717
8	Skewness	+0,542995584	0,270688958
9	Range	0,08	0,18
10	Minimum	0,64	0,61
11	Maximum	0,72	0,79
12	Sum	3,42	3,44
13	Count	5	5
14	Confidence level (95%)	0,037865741	0,101026192

The standard deviation's little values indicate the fact that the data are homogenous around mean values.

We have tested the hypothesis if the total alkaloid content is the same at ergocristine and ergotamine strains in the case of a 95% confidence level (table III).

Table III. t-test for CAT at ergocristine and ergotamine strains

Crt. No	Statistical indicator	Ergocristine strains	Ergotamine strains
1.	Mean	0,684	0,688
2.	Variance	0,00093	0,00662
3.	Observations	5	5
4.	Hypothesized Mean Difference	0	
5.	df	5	
6.	t Stat	**-0,102937003**	
7.	P(T<=t) one-tail	0,461007024	
8.	t Critical one-tail	2,015049176	
9.	P(T<=t) two-tail	0,922014049	
10.	t Critical two-tail	**2,570577635**	

The tested hypothesis indicates the fact that the total alkaloid content is the same indifferent the tested strain alkaloid content.

The results of total lipids determinations are presented in table IV.

In the total lipids determination there are not errors (table V) and we tested further on the hypothesis of the 4g difference between the ergocristine and ergotamine type strains in the case of 95% confidence level (table VI).

Table IV. The total lipid content of ergocristine and ergotamine type *Claviceps purpurea* sclerotia

Crt. No.	Strain type	Total lipids (g%)
1	S1	31,46
2	S2	39,00
3	S3	32,41
4	S4	41,26
5	S5	32,74
6	T1	36,35
7	T2	35,84
8	T3	41,63
9	T4	41,35
10	T5	40,94

Table V. Statistical analysis of the total lipid content of ergosristine and ergotamine type *Claviceps purpurea* sclerotia

Crt. No.	Statistical indicator	Ergocristine strains	Ergotamine strains
1	Mean	35,374	39,222
2	Standard error	1,985390642	1,283835659
3	Median	32,74	40,94
4	Mode	#N/A	#N/A
5	Standard deviation	4,439468437	2,870743806
6	Sample variance	19,70888	8,24117
7	Kurtosis	-2,422744943	-3,183416241
8	Skewness	0,701549855	-0,593565729
9	Range	9,8	5,79
10	Minimum	31,46	35,84
11	Maximum	41,26	41,63
12	Sum	176,87	196,11
13	Count	5	5
14	Confidence level (95%)	5,512339546	3,564506615

Table VI t-test for total lipid content at ergocristine and ergotamine strains

Crt. No.	Statistical indicator	Ergotamine strains	Ergocristine strains
1	Mean	39,222	35,374
2	Variance	8,24117	19,70888
3	Observations	5	5
4	Hypothesized Mean Difference	4	
5	df	7	
6	t Stat	-0,064289093	
7	P(T<=t) one-tail	0,475268717	
8	t Critical one-tail	1,894577508	
9	P(T<=t) two-tail	0,950537434	
10	t Critical two-tail	2,36462256	

The tested hypothesis indicates that total lipid content is different for the two analysed alkaloid types. The mean difference between ergotamine and ergocristine types is 4g.

To find weight average we have considered 20 sclerotia for each determination. The results are presented in table VII.

Table VII The weight of ergocristine and ergotamine type *Claviceps purpurea* sclerotia

Crt. No.	Strain type	Weight (g/sclerotium)
1	S1	0,05718
2	S2	0,04078
3	S3	0,05600
4	S4	0,07625
5	S5	0,06718
6	T1	0,09379
7	T2	0,07388
8	T3	0,09393
9	T4	0,07444
10	T5	0,07375

We have statistically analysed the results and we have concluded the homogeneousness around the mean:

Table VIII Statistical analysis about the weight of ergocristine and ergotamine type *Claviceps purpurea* sclerotia

Crt. No.	Statistical indicator	Ergocristine strains	Ergotamine strains
1	Mean	0,061268	0,081958
2	Standard error	0,00466824	0,004860405
3	Median	0,05718	0,07444
4	Mode	#N/A	#N/A
5	Standard deviation	0,10438502	0,010868195
6	Sample variance	0,000108962	0,000118118
7	Kurtosis	-0,630480519	-3,329126646
8	Skewness	0,668844587	0,606094432
9	Range	0,02647	0,02018
10	Minimum	0,04978	0,07375
11	Maximum	0,07625	0,09393
12	Sum	0,30639	0,40979
13	Count	5	5
14	Confidence level (95%)	0,012961139	0,013494675

We have tested the hypothesis that the indicator has comparable values at ergocristine and ergotamine strains with a 95% confidence level (table IX).

Table IX t-test for the weight of ergotamine and ergosristine type *Claviceps purpurea* sclerotia

Crt. No.	Statistical indicator	Ergocristine strains	Ergotamine strains
1	Mean	0,061278	0,081958
2	Variance	0,000108962	0,000118118
3	Observations	5	5
4	Hypothesized Mean Difference	0	
5	df	8	
6	t Stat	-3,068641142	
7	P(T<=t) one-tail	0,007689791	
8	t Critical one-tail	1,85954832	
9	P(T<=t) two-tail	0,015379583	
10	t Critical two-tail	2,306005626	

The test's results indicate that the ergocristine sclerotia weight is different from ergotamine sclerotia weight in favour of ergotamine type.

SUMMARY

In this paper we present the results concerning the correlation between total lipids content and biosynthetic capacity expressed by total alkaloid content. We have analysed ten dried *Claviceps purpurea* sclerotia of ergotamine and ergocristine types, harvested from rye artificial infected. At ergotamine strains the total lipids amount is higher than the ergocristine strains, behaviour that is not present at the biosynthetic capacity. We find also that the ergotamine strains studied present a higher weight than the ergocristine type.

CONCLUSIONS

The alkaloids and lipids have common precursors and parallel biosynthetic way for these compounds. Our study praise that at comparable biosynthetic alkaloid capacity ergotamine strains has a total lipid content higher than ergocristine strains. In concordance with these results is the weight of ergotamine and ergocristine type *Claviceps purpurea* sclerotia which is also higher at ergotamine type.

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