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8. Testing some extracts of plants from the Danube Delta with potential antiparasitic effect on equines

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Abstract: The study was done during november 2016 on strongyls eggs from feces from a total of 28 horses in Tulcea county, C.A. Rosetti locality in the Danube Delta. To determine the degree of infestation was used the method McMaster and Stoll (adapted for quantitative larvohelmintology). The tests of hatching eggs (egg hatching assay - EHA) and larval development (larvae development assay - LDA) were made, using as active substances herbal decoctions and hydro-alcoholic solutions made from well known anthelmintic plants.
One decoction and two hydro-alcoholic extracts were tested, *Hippophae rhamnoides* (sea buckthorn) respectively *Thymus serpyllum* (creeping thyme) and *Arthemisia absinthium* (wormwood). In order to achieve the two tests for therapeutic efficacy of the tested products were obtained six serial dilutions with concentrations of 50%, 25%, 12.5%, 6.25%, 3.12%, and 1, 56%. For decoctions were made control samples with distilled water, and with alcohol for hydroalcoholic solutions. The data obtained in both tests were analyzed using Anthelmintic Rezistance Program (ARP), determining the parameters "a" and "b" to trace the line prediction and lethal concentrations (CL). We can be concluded that for the decoction was observed an emphatic decrease of the percentage of larval development. For the two hydro-alcoholic extracts the hatching percentage of Strongyls eggs was different depending on the concentration used, the maximum value being registered at 25% dilution, in which the percentage of hatching was 82.35%, and the minimum concentration of 12.50%. LDA test showed a significant reduction in the percentage of larval Strongyls development, stage 3, at all dilutions, which is less than 50%.

Key words*:* strongylidosis, plant extract, parasitology, extracts testing, antiparasitic effect, Danube Delta

INTRODUCTION

The concerns and achievements of knowledge and combating parasitosis in equines have not yet achieved the needs and expectations in most countries. The equines, this species so important in a full era of automation and cybernetics and of the evolution of the market economy, has not yet lost its place in many areas of agriculture, transport, sport, art, etc., due to its traction capacity, aptitude and behavior. It is also recognized the past, present and future important role of equines in human history, within the ecosystems and their balance, due to their biological and morphofunctional qualities, so useful to society.[Şuteu, 1994].

The study of strongylidosis in equines is internationally topical because these diseases can have serious repercussions caused both by its symptoms but especially by the decreased physical capacity of animals. Thus, the presence of strongyls can alter the behavior, fertility, fitness, body development to adulthood, decreases the resistance to other pathogens and could become even death cause.

At the beginning of the 70's, following the introduction of the broad-spectrum anthelmintics, it was found a significantly decrease of the rate of infestation of the equines population by helminths. The additional hygiene measures designed to prevent reinfestations, have also contributed to this decrease. However, parasitism still play an important role in the modern management of equine breeding. Worldwide, the epidemiological situation of strongylidosis reveals that the strategies on combating aimed at reducing the pathogenicity and the economic consequences and limiting the infestations, but the eradication is not possible yet [Bussieras and Chermette, 1995].

The resistance to anthelmintic has gradually evolved from a veterinary medical curiosity to an important economic issue. The anthelmintic pharmacology and the resistance to these drugs has been studied more in the nematodes parasitizing the sheep. Anthelmintic resistance in recent years has become a particularly serious problem worldwide, many species of nematodes becaming resistant, especially in sheep, goats and equine [Bauer and colab., 1986; Bjørn and colab., 1991; Boersema and colab., 1991; Amarante and colab., 1997; Cristina and colab, 1999; Kaplan, 2002; Cosoroabă, 2002; Didă and colab., 2002].

Taking into account the global situation of the chimio-resistance of the equine strongyls, it is currently trying to find new methods to counteract this phenomenon, phytotherapy being one of the possible alternatives. Strongylidosis are geohelmintoze cosmopolitan species of the family *Strongylidae*, parasitizing at mammals, marsupials, birds

and reptiles. For the equine species are known over 60 species of strongyls [Lichtenfels şi colab. 1998] determining disease forms with subclinical, clinical, acute or chronic evolution, manifested by digestive disorders, anemia and weight loss. A common clinical sign in horses with strongylidosis is the colic syndrome, whose intensity varies from mild to violent. [Şuteu şi Cozma, 1998].

MATERIALS AND METHODS

The study was done during November 2016 on strongyls eggs from the excrements from a number of 28 horses in Tulcea county, C.A.Rossetti and Letea localities (**Table 1**). To determine the degree of infestation, the McMaster and Stoll method (adjusted for quantitative larval helmintology) was used (**Table 1**). Thereafter, the strongyls eggs were collected from the fecal samples using the Willis flotation method.

Table 1

The average egg infestation (OPG) and larvae (LPG) of equines strongyls from the C.A. Rossetti and Letea localities, Tulcea county (June - September 2016)

Sample Number	Sex	OPG	LPG
1	F	100	200
2	F	1.000	2.200
3	F	1.900	2.000
4	F	500	900
5	F	300	1.100
6	F	500	700
7	F	2.800	4.100
8	F	400	1.000
9	F	100	600
10	F	0	100
11	F	0	100
12	F	0	200
13	F	100	600
14	F	300	600
15	F	600	1.100
16	M	300	1.100
17	F	1.000	2.300
18	F	800	800
19	F	100	800
20	F	0	500
21	F	100	400
22	F	400	500
23	F	1.500	2.200
24	F	1.000	1.600
25	F	5.800	8.300
26	M	1.400	1.900
27	F	2.800	3.300
28	F	0	700

The tests of hatching eggs (egg hatching assay - EHA) and larval development (larvae development assay - LDA) were made, using as active substances herbal decoctions and hydro-alcoholic solutions made from well known anthelmintic plants. One decoction and one hydro-alcoholic extract were tested (**Table 2**).

Table 2

The composition of the decoctions and herbal solutions used in EHA and LDA tests as well as the pharmaceutical form

No.	composition (plant)	Pharmaceutical form
1	*Hippophae rhamnoides* (sea buckthorn)	Decoction
2	*Arthemisia absinthium* (absinth)	Hydroalcoholic solution

In order to perform these tests decoctions were prepared according with the Romanian Pharmacopoeia, using 6 grams of vegetable per 100ml of water, representing the "mother" solution. For the hydroalcoholic solutions, it was not necessary to make any preparations in advance. In order to achieve the two tests for the emphasizing of the therapeutic efficacy of the tested products, six serial dilutions were obtained, with concentrations of 50%, 25%, 12.5%, 6.25%, 3.12%, and 1, 56%. The control samples (witness) were made with distilled water for decoctions, and with alcohol for the hydroalcoholic solutions.

For the EHA test, the samples were kept in an incubator at 26°C for 48 hours, after which the results were read, quantifying the number of strongyls morula eggs compared with the hatched eggs and the stage 1 larva (L1). It was calculated the percentage of hatching and was drawn the prediction line for each sample, separately. For LDA test, the reading of the results was performed after 12 days of incubating the plates in a thermostat, quantifying the morula eggs and the larvae of stage 1 and 2 (L1, L2) compared to those of stage 3 (L3). It was determined the percentage of larval development and the equation of the prediction.

Data obtained in both tests were analyzed using the Anthelmintic Rezistance Program (ARP), determining the parameters "a" and "b" to trace the prediction line and lethal concentrations (LC) 50, 90, 100.
Metoda McMaster. The parasitic elements (eggs) dispersed in a saturated solution of sodium chloride, float on the surface of the counting room, where they will be counted and expressed per gram of excrements (OPG).

Counting eggs is made watching the grid of the 6 rectangles in each room, and if the eggs are at the edge of the room, the counting is made only for these on the two sides/edges of the room. The number of eggs per gram of excrements (OPG) is obtained by applying the following formula:

$$OPG = [(N1 + N2) / 2] \times 100 \text{ } or \text{ } OPG = (N1 + N2) \times 50$$

where: N1 represents the number of eggs counted in the first room

N2 represents the number of eggs counted in the second room

The Stoll Method (adapted) – for quantitative larvohelmintoscopy., intended to quantify the larvae from excrements recently collected. [Thienpont şi colab., 1986]. This method was adapted for the coprocultures excrements, to quantify the strongyls larvae.

Required materials: 100ml graduated tube, a rubber stopper, pharmaceutical balance, solution of sodium hydroxide 4 ‰ (0.1 N), water, glass pearls, automated pipettes, microscope lamellas (or microscope lamellas with buckets) and microscope.
Work technique: 5g of excrements from stool are weighting and than are placed in a graduated tube. The sodium hydroxide 4 ‰ (0.1N) solution is added over it up to 75ml graduation. In order to facilitate the homogenization of the excrements some glass pearls are inserted in the test tube. The tube is closed with a rubber stopper and it is shaked vigorously until a homogeneous solution is obtained. Immediately an amount of 0.15ml is taken with an automatic pipette, and then this amount is put on a microscope lamella (or on a microscope lamella with buckets) and is examined all with a 10 x lens. 3-4 readings of each sample are made for accuracy. To get the number of larvae per gram of excrements (LPG), the following formula is applied:

$$LPG = TN \times (75 : 5) \times (1 : 0{,}15) \text{ } or \text{ } LPG = TN \times 100$$

unde: TN = total number of the counted larvae
75 = represents ml of sodium hydroxide 4‰ (0,1N) solution
0,15 = represents ml taken from 75ml
5 = represents the quantity of examined excrements, in grams

Strongyls eggs collection. It is an adaptation of the Willis method, aimed at obtaining of a sufficient number of strongyls eggs from anaerobic samples, necessary for testing *in vitro* of the chemoresistance [Coles et al., 1992]. The principle is based on the floating of light strongyls eggs on the surface of the saturated sodium chloride solution, and their adherence to a glass surface.
Used materials. Magnetic stirrer, sieves, Berzelius glasses, centrifuge tubes, pipettes, centrifuge, saturated sodium chloride solution, distilled water.
Work technique. The excrements samples were homogenized with a magnetic stirrer filtered afterwards through a successively set of sieves with a mesh size of 100µm, 50µm and 20µm. The residue on the sieve with a mesh size of 20 µm was collected in a fine spray of distilled water in a Berzelius glass. The obtained liquid was centrifuged for 2 minutes at 3000 rpm then the supernatant was removed. Over residue was added a saturated solution of sodium chloride until a superior meniscus was formed. At the top of the centrifuge tube a microscope lamella was applied to contact with the formed meniscus, avoiding the formation of air bubbles. The tubes prepared in that manner were allowed to stand for 20 minutes then the lamella was removed from the glass. The liquid adhering to the lamella was collected in a Berzelius glass, by washing it under a fine spray of distilled water. The last two steps were repeated several times, until a sufficient number of eggs have been collected. Finally, the liquid collected in the Berzelius glass was placed in a centrifuge tube graduated with a capacity of 12ml, it was filled with distilled water up to 10ml and it was centrifuged for 5 minutes at 3000 rpm, then 5ml of the supernatant were aspirated. The remaining liquid in the tube was shaken, 50µl was aspirated and then examined under a microscope by counting all eggs from the sample.

Total number of eggs per mililitre was obtained applying the formula: Nr.of eggs per ml = (1000 x nr. eggs per 50µl) / 50

Egg hatch assay - EHA is a collective term that designates a number of methods used to detect resistance to benzimidazole. All are based on ovocide properties of benzimidazole and the ability the eggs from the resistant strains have, to embryon and to hatch at higher concentrations at anthelmintics than the eggs from sensitive parasites. The principle of these tests lies in the eggs' incubation in serial dilutions of a benzimidazole and then counting the proportion of eggs that were unable to embryonate and / or hatching. I adapted this test to determine the lethal concentrations at each tested vegetable product.

Starting with Le Jambre [1976], this method has been modified or adapted by different authors [Coles et al., 1977]. The various procedures described above were reviewed by Johansen [1989]. The incubation temperature is generally around 26°C and the reading of the results is carried out after 24, 48 or 72 hours. At this point, the LD_{50} and the resistance factor of the studied strain is determined. Egg hatch assay (EHA) uses TBZ property to inhibit the nematode's eggs to embryo and hatching, thus calculating the lethal dose 50 (LD_{50}) of the drug. This assay is suitable to be used for the species of nematodes whose eggs are hatching quickly [Coles et al., 1992; Ullrich, 1987; Craven et al., 1999]. The interpretation of the results at the Egg Hatch Assay (EHA) is made upon a reference dilution of 0,15µg / ml TBZ, considering as being resistant that strongyls populations of which the eggs hatched at a rate greater or equal to 50%.

If the number of larvae and eggs of a test is very high we can examine only a quantity of 0.5ml then the result may be multiplied by two. Hatching percentage is calculated according to the formula:

% hatching = [(EE + L1) / (E + (EE + L1)] x 100

where: EE = embryonated eggs
L1 = first stage larvae
E = unembryonated eggs (morulated)

Larval evelopment assay - LDA. This test was designed and developed by Coles et al. [1988] and subsequently modified by Taylor [1990] and is based on breeding of *Trichostrongylidae* larvae on a nutrient medium. In such environment, from the strongyls eggs are hatching L1 larvae, that within 6-10 days are growing up to the point of infesting L3 larvae. Adding antihelmintics in various dilutions allows highlighting the existence of resistant strains, capable of growth at anthelmintics lethal concentrations to a sensitive reference strain. The result is expressed as a lethal concentration necessary to prevent larval development. The percentage of larval development needed to determine CL is calculated according to the formula:

% larval development = [L3 / (E + L3)] x 100

where: L3 = third stage larvae (infesting)
E = unembryonated eggs (morulated)

To highlight the therapeutic efficacy of the extracts of the tested plants, the following parameters were determined:
- the percentage of hatching or larval development;
- the prediction line and its equation;
- the lethal concentration 50, 90, 100;

For the accuracy, the correlation and the correct interpretation of the data obtained both for EHA and for LDA tests, a computer software called Anthelminitic Resistance Program (ARP) was used. The results' interpretation at the classic EHA test is made to a reference dilution of 0,15µg / ml of thiabendazole, considering as resistant that strongyls populations to which the eggs hatched at a higher or equal rate to 50% [Coles et al.,1992; Requejo-Fernandez et al., 1997; Madeira de Carvalho, 2001]. With this software we can automatically calculate the lethal concentration 50, 90 or 100 (LC_{50}, LC_{90} or LC_{100}), regardless the test we use. In the performed tests we observed that the percentage of hatching or larval development does not increase progressively with the decreasing of the tested substance's concentration. Therefore, the interpretation of the test is inadequate and insufficient if it is based only on the percentage of hatching at a certain concentration or if it based on the concentration to which the first third stage larvae appear, and if there are not taken into consideration the results obtained at lower or higher concentrations than this. Through ARP software, these interpretation errors of the obtained data are fully eliminated.

LC_{50} calculation for the studied active substances, requires, initially, the obtaining of a prediction line, estimated in terms of a first rank polynomial (ax + b). For this, it was appreciated the dynamic of the hatching percentage at different concentrations of tested extracts within the range of 50 and 1.56%. The dynamic evolution of the coordinates, which shows the hatching percentage in various concentrations of active substance, contributes to the overall statistical estimation of action of these extracts on eggs hatching.

The calculation of the LC_{50} requires, in the first instance, the drawing of a prediction line, and then, based on its parameters (a and b) we can interpret the data relating to the different tested concentrations. The prediction line is an average of all the lines that pass through every two points on the diagram (dilutions representing the abscis axis and the hatching percentage the ordinate axis).

RESULTS AND DISCUSSIONS

The chemical examination of *Hippophae rhamnoides* (seabuckthorn), led to highlighting the groups of active ingredients, which gives it an important potentially therapeutic role: volatile oil in flowers; resin acids in flowers, young branches and buds; carotenoids in flowers, young branches, buds and fruits; reducing compounds (flavonoids, polyphenolcarboxilics acids and oses) in all investigated plant products; tannins in all investigated vegetable products, less in buds, its dynamics of accumulation depending on maturity ranging body; flavonosids in flowers, leaves, young branches, buds and fruits; proantocianosides in fruits; mucilages in flowers, young branches, buds and fruits and simple oses in fruits [Bodea şi colab., 1997]. The fruits contain many valuable vitamins, so the seabuckthorn has twice more vitamin C than the dog-rose, previously known as the richest in this vitamin. The content of carotene (provitamin A), citric acid and mannite is 10 to 15 times higher than in the lemon (550-900mg per 100g of juice), and the vitamin E which is revitalizing the human body is found in the seabuckthorn oil in an higher amount (200 mg %) than in all fruit-growing species and more than in soybean (120 mg %), corn (100 mg %) and sunflower (100 mg %). In the seabuckthorn fruits are also found vitamins like P, B1, B2, A, K, M, etc. In the seabuckthorn fruits were identified also 15 microelements (iron, manganese, boron, aluminum, titanium, etc.)

[Mincu, 1993]. The beneficial effects of this plant are known since antiquity. Currently, the following products are obtained from the seabuckthorn: tea made of fruits, buds, leaves and even bark, fruit syrups, fruit oil. The latter is the most valuable in terms of medical properties, having anthelmintic compounds. The seabuckthorn oil is used in the treatment of some medical conditions such as gastric and duodenal ulcers, allergies, diarrhea, rash, rheumatism or neuroendocrinological, circulatory and liver diseases. It is used in geriatrics, too with spectacular results. With buckthorn are also treated ophthalmic and coronary diseases, arterial hypertension and gingivitis. By processing it in pharmaceutical laboratories, from the seabuckthorn are obtained extraordinary treatments for treating: depressions, Parkinson's disease, tumors, adenomas and leukemia [Brad, 2002].

The seabuckthorn fruit is primarily a natural polyvitamin, with special recommendations for convalescents in weakness status, toning both children and the elderly. Especially in winter, when the resources of natural vitamins are low, the infusion of buckthorn, the syrup and the jam are welcomed and can be used every day as tea food, especially due to the pleasant aroma of the fruit. Particularly important are the effects on the circulatory system given by the flavones. They increase the myocardial contractile force, probably due to a better coronary circulation and lead to the improvement of the overall metabolism. The quercetrina mainly involve a vasoconstriction and a decreased capillary permeability, which explains its effects on the hemorrhagic diseases, such as purpura. The vasoconstriction is followed by a vasodilatation in the kidney, increasing diuresis, so the fruits and preparations of fruits are being useful in renal insufficiency and inedome. Adding the blood pressure lowering effect, it leads to a complex and toning action on the circulatory system, different from that of digitalis or caffeine, being useful in mild cardiac insufficiencies or even in severe forms if associated with the basic drugs. The infusion of buckthorn it is also recommended in diarrhea, rheumatism, but in particular as anthelmintic.

The pharmacodynamic assays performed with seabuckthorn bark decoction and seeds, using EHA test, revealed its poor efficacy on the strongyls eggs from horses. It was noted that even at the maximum concentrations (50%) the percentage of hatching was 98.59%, the minimum being paradoxically recorded at the minimum concentration of 2.44% (**Table 3**).

Table 3

Statistical indices obtained at EHA test by using the seabuckthorn decoction in the case of dejections samples of the horses from C.A. Rossetti and Letea localities, Tulcea county (June – September 2016)

Sample no.	No. of eggs	No. of embryonated eggs +L1	Dilution percentage	Hatching percentage	Equation (prediction line)
1	100	7000	50,00	98,59	-101,83
2	1200	1500	25,00	55,56	-35,83
3	2800	400	12,50	12,50	-2,83
4	2100	1600	6,25	43,24	13,67
5	2800	400	3,12	12,50	21,93
6	4000	100	1,56	2,44	26,05
Control sample (Witness)	400	3100	0	88,57	30,17

Parameters		LC_{50}	LC_{90}	LC_{100}
a	-2,64	-7,4830	-22,5796	-26,3538
b	30,17			

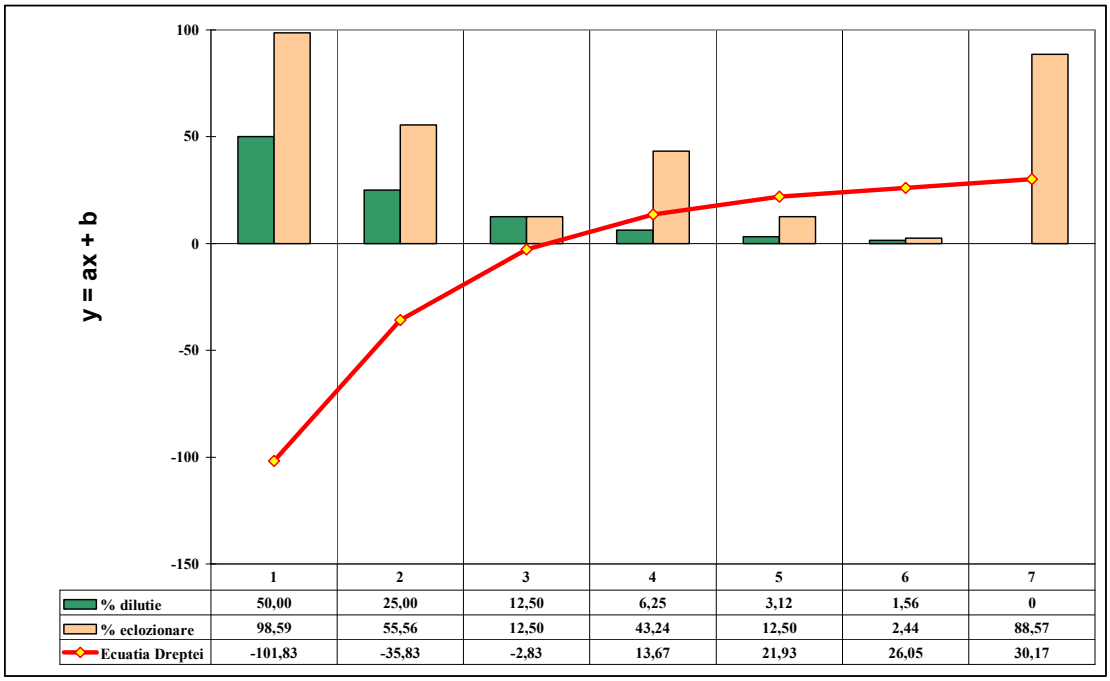


Fig.1 The aspect of the prediction line in the case of the EHA testing of the de seabuckthorn decoction on the strongyls eggs of the horses from C.A. Rossetti and Letea localities, Tulcea county (June – September 2016)

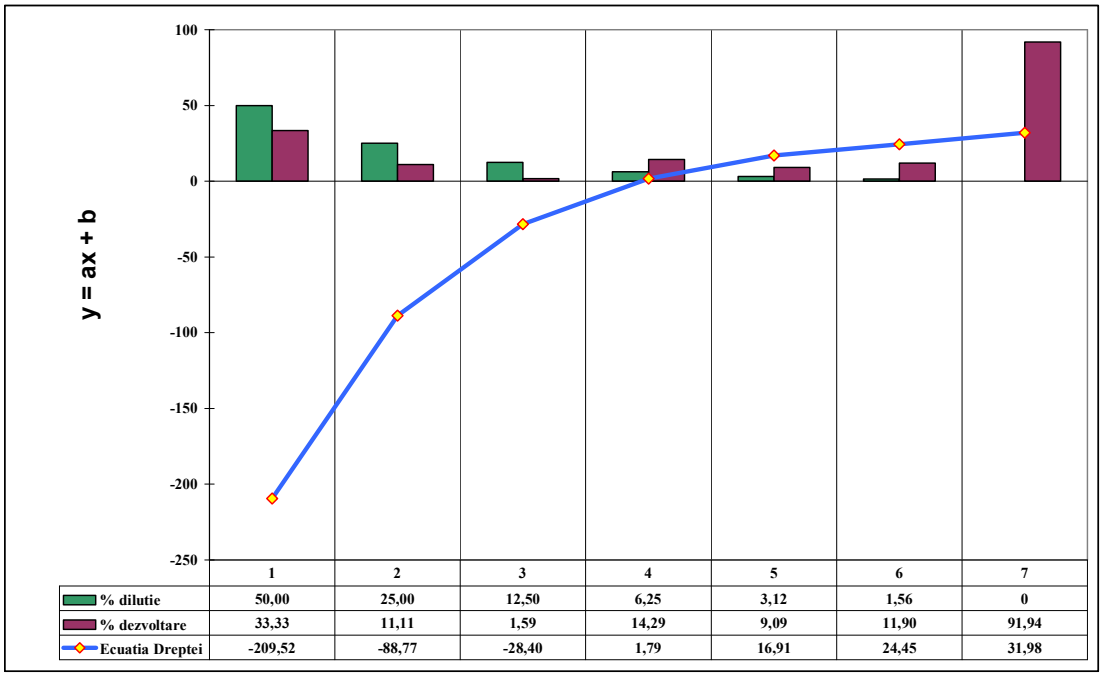
Conversely, at the larval development test (LDA), carried out with the same decoction of sea buckthorn, a strong decrease of the percentage of larval development has been observed. It had a minimum of 1.59 % at a concentration of 12.50 % and a maximum of 33.33 % at the concentration of 50 % (**Table 4**). It was noted a similar situation to that encountered at EHA test, which showed that the seabuckthorn concentrated decoction is less effective than the more diluted liquids. Drawing the line prediction showed a negative trend to its minimum value reaching -209.52. The 50 lethal concentration is much lower than the one determined in the previous test, reaching -3.7261%, with CL_{100} also diminished (-14.0665) (**Table 4**). This phenomenon highlights the fact that the extended contact of exogenous parasitic forms of strongyls and the active compounds of seabuckthorn decoction caused an increase in the effectiveness of this pharmaceutical product.

Table 4

Statistical indices obtained at LDA test by using the seabuckthorn decoction in the case of dejections samples of the horses from C.A. Rossetti and Letea villages, Tulcea county (June – September 2016)

Sample No.	No. of eggs, L1, L2	No. L3	Dilution percentage	Development percentage	Equation (prediction line)
1	800	400	50,00	33,33	*-209,52*
2	3200	400	25,00	11,11	*-88,77*
3	6200	100	12,50	1,59	*-28,40*
4	3600	600	6,25	14,29	*1,79*
5	3000	300	3,12	9,09	*16,91*
6	3700	500	1,56	11,90	*24,45*
Control sample (Witness)	500	5700	0	91,94	*31,98*

Parameters		LC_{50}	LC_{90}	LC_{100}
a	-4,83	-3,7261	-11,9984	-14,0665
b	*31,98*			



	1	2	3	4	5	6	7
% dilutie	50,00	25,00	12,50	6,25	3,12	1,56	0
% dezvoltare	33,33	11,11	1,59	14,29	9,09	11,90	91,94
Ecuatia Dreptei	-209,52	-88,77	-28,40	1,79	16,91	24,45	31,98

Fig. 2 The aspect of the prediction line in the case of the LDA testing of the de seabuckthorn decoction on the strongyls eggs of the horses from C.A. Rossetti and Letea localities, Tulcea county (June – September 2016)

In order to determine the therapeutic efficacy of various plant extracts through *in vitro* tests, three hydroalcoholic commercial preparations of the thyme, basil and wormwood plants were tested. The testing of the pharmacodynamic effect on strongyls eggs of horses was also conducted by the two established tests: the test of egg hatching (EHA) and larval development test (LDA).

Hydroalcoholic solution from wormwood

The plant has therapeutic use in human and veterinary medicine, both modern and traditional. Human medicine use the plant for internal usage: to stimulate the appetite, removing of some toxic substances from the body, treating hypertension, eliminate intestinal worms, removing feelings of nausea, in kidney edema, stomach hypoacidity, dyspepsia with constipation, to combat intestinal worms, to improve digestive activity, in biliary dyskinesia, for treating stomach, liver, menstrual cycle disorders, to treat fever swamp (malaria). In external use: to combat pinworms, for the treatment of the festering wounds, ulcers. Veterinary medicine use the wormwood to treat the indigestion in the prestomachs inertia. In overdose, the plant causes in animals toxic conditions manifested by amaurosis, general excitement, anxiety, fear, sudden increase in temperature, convulsive contractions, muscle tremors, clonic convulsions, opistotonus, sweating. Acute evolution lasts 3-5 hours, ending with death.

In the case of the hydro-alcoholic solution of wormwood, the percentage of hatching of the strongyls eggs was different, depending on the concentration used, the maximum being recorded at 25 % dilution, where the percentage of hatching was 82.35%, while the minimum was recorded at 12.50 % concentration (0%) (**Table 5**).

Table 5

Statistical indices obtained at EHA test by using the hydro-alcoholic solution of wormwood in the case of dejections samples of the horses from C.A. Rossetti and Letea localities, Tulcea county (June – september 2016)

Sample no.	No. of eggs	No. of embryonated eggs +L1	Dilution percentage	Hatching percentage	Equation (prediction line)
1	1100	2700	50,00	71,05	*-163,54*
2	300	1400	25,00	82,35	*-67,04*
3	3800	0	12,50	0,00	*-18,79*
4	5100	600	6,25	10,53	*5,34*
5	3500	200	3,12	5,41	*17,42*
6	3200	600	1,56	15,79	*23,44*
Control sample (Witness)	400	3100	0	88,57	*29,46*

Parameters		LC_{50}	LC_{90}	LC_{100}
a	-3,86	-5,3128	-15,6600	-18,2467
b	*29,46*			

These values, combined with a percentage of hatching of 88.57% achieved in the witness sample with alcohol and water, led to negative lethal concentrations CL_{50} of -5.3128%. The minimum value of the parameter Y was only -163.54 (**Fig. 3**).

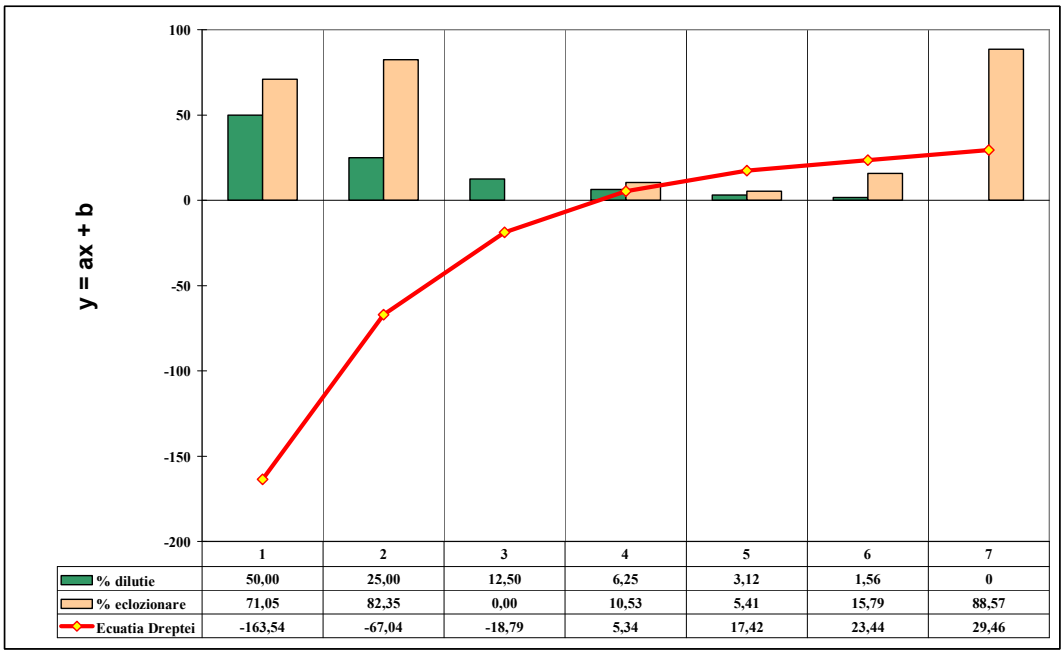


Fig. 3 The aspect of the prediction line in the case of the EHA testing of the hydro-alcoholic solution of wormwood on the strongyls eggs of the horses from C.A. Rossetti and Letea localities, Tulcea county (June – September 2016)

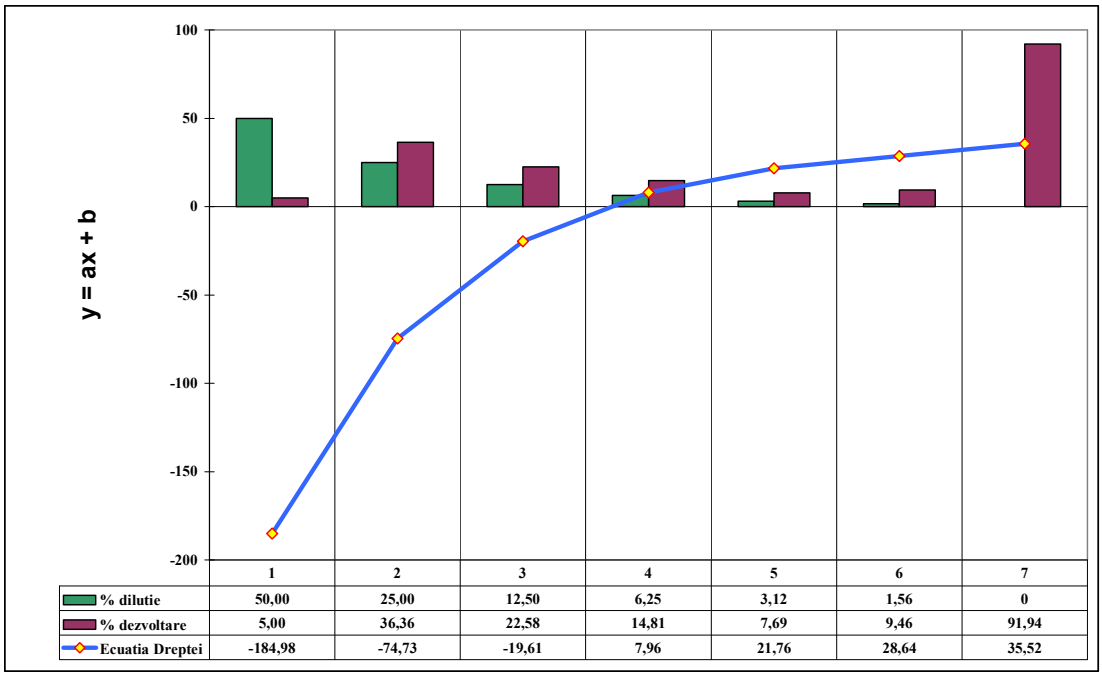
In the case of the LDA test, the maintenance of the samples for 12 days at the 26^0C temperature resulted in a significant reduction in the percentage of the development of the stage 3 larval strongyls. Practically, at all dilutions, the percentage of larval development was less than 50%, the minimum being recorded at the maximum concentration of 50% where was found a total of 100 individuals of larvae stage 3. This good efficacy of the hydro-alcoholic solution of wormwood is emphasized by the value of CL_{50}, too, which was -3.2751%. The statistical analysis (by ARP) of the data obtained from LDA tests, revealed a high percentage of larval development in the witness sample, with a value of 91.94%, which demonstrates that the hydro-alcoholic excipients of the product do not significantly affect the percentage of the larval development of the stage 3 larvae (**Table 6**). The ARP determination of the prediction line showed a negative trend based on the value of 35.52 and reaching a minimum parameter of -184.96 Y (**Fig. 4**). It thus demonstrate the better efficacy of the hydro-alcoholic extracts on the strongyls eggs and larvae if the period of contact between them and the plant active elements is longer.

Table 6

Statistical indices obtained at LDA test by using the hydro-alcoholic solution of wormwood in the case of dejections samples of the horses from C.A. Rossetti and Letea localities, Tulcea county (June – September 2016)

Sample no.	No. of eggs, L1, L2	No. L3	Dilution percentage	Development percentage	Equation (prediction line)
1	1900	100	50,00	5,00	-184,98
2	700	400	25,00	36,36	-74,73
3	2400	700	12,50	22,58	-19,61
4	4600	800	6,25	14,81	7,96
5	4800	400	3,12	7,69	21,76
6	6700	700	1,56	9,46	28,64
Control sample (Witness)	500	5700	0	91,94	35,52

Parameters		LC_{50}	LC_{90}	LC_{100}
a	-4,41	-3,2751	-12,3266	-14,5894
b	35,52			



	1	2	3	4	5	6	7
% dilutie	50,00	25,00	12,50	6,25	3,12	1,56	0
% dezvoltare	5,00	36,36	22,58	14,81	7,69	9,46	91,94
Ecuatia Dreptei	-184,98	-74,73	-19,61	7,96	21,76	28,64	35,52

Fig. 4 The aspect of the prediction line in the case of the LDA testing of the hydro-alcoholic solution of wormwood on the strongyls eggs of the horses from C.A. Rossetti and Letea localities, Tulcea county (June – September 2016)

CONCLUSIONS

The *in vitro* phytotherapeutic researches carried out during June-September 2016, on the excrements samples from a total of 28 horses in Tulcea county, CARossetti and Letea localities, in order to determine the effectiveness of a plant decoction and a plant hydro-alcoholic solution on the strongyls eggs have revealed the following:

- decoction of seabuckthorn: in EHA test, at the peak concentrations (50%) the percentage of hatching was 98.59%, with the minimum being paradoxically recorded at the minimum concentration of 2.44%; by contrast, in LAD test there was a sharp drop in the percentage of larval development with a minimum of 1.59% at a concentration of 12.50% and a maximum of 33.33% at the concentration of 50%, emphasizing the fact that by extending the duration of the contact between the exogenous parasitic forms of the strongyls and the active elements of the seabuckthorn decoction caused an increase in the effectiveness of this pharmaceutical product.
- hydro-alcoholic solution of wormwood: the percentage of hatching of the strongyls eggs was different, depending on the concentration used, the maximum being recorded at 25% dilution, where the percentage of hatching was of 82.35%, while the minimum was recorded at the concentration of 12.50% (0%); LDA test showed a significant reduction in the percentage of larval development of stage 3 strongyls larvae at all dilutions, which is less than 50%, with a LC_{50} of -3.2751%.

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