https://doi.org/10.31871/IJNTR.6.2.17
International Journal of New Technology and Research (IJNTR)
ISSN: 2454-4116, Volume-6, Issue-2, February 2020 Pages 12-16

Use of Chelate Zinc in Vermiculture

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Abstract— **The impact of glutamine acid (GlH2)-containing chelate zinc on earthworms' protein mass and the degree of cocoon reproduction is studied, and the effect of different external stress-factors: inundation, substrate quality and chelate zinc toxic doses on earthworms is established, as well. For this purpose, an experiment was conducted according to one and the same pattern by four options. Four groups (one control and three test ones with maximum (Max), normal (Norm.) and minimum (Min.) doses in grams) were composed for all options. Each of them has three repetitions. 5 earthworms of roughly equal mass were selected for each repetition. Weighing and cocoons' counting was made in three stages, on the 21st, 31st and 41st day since the start of the experiment. Earthworms' mass gain was registered in all groups throughout the course of test in the first option of the experiment. At that, mass gain degree in all test groups was quite high compared to the control one and reached the maximum in II test group – 108.6%. Reproduction growth degree compared to control group was higher in all test groups and reached the maximum in I test group – 152.9%. Based on the analysis of obtained results the optimum and effective dose of chelate zinc is established and it equals to 0.0269g. per 300g. of substrate.**

When studying external stress factors – inundation (second option) and poor-quality substrate (third option), on the basis of conducted studies an enhancement of resistance of earthworms treated with chelate zinc to different external stress factors is established. All earthworms of all three repetitions of the control group died, while in the test group – only part of them. On the basis of experiment conducted on earthworms (fourth option) treated with chelate zinc one can draw conclusion that they are quite sensitive to high doses of microelements, earthworms of all test groups died, while all five earthworms of all repetitions of the control group were alive. Thus, earthworms can be successfully used as toxicity biotests, and that is very important due to cheapness and simplicity of this method.

We suppose that obtained results are of great importance for agriculture, particularly in vermiculture, since earthworms breeding and use of this renewed bioresource for obtaining high-quality vitamin-proteinic feed premixes in poultry, animal farming and fishing is one of its important direction.

Index Terms— **Vermiculture, Earthworm, Cocoon, Microelement, Substrate, Reproduction degree.**

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I. Introduction

The mankind enters a new millenium facing serious problems of starvation and insufficient nutrition. Of course, these acute problems are on the agenda in our country, as well. Based on this fact, population provision with high-quality, ecologically safe and cheap agricultural products is very topical today [1-8]. That's why earthworms are destined to play important role in solution of this problem [9-16]. So, a study of effect of different factors on earthworms' protein mass gain and increase of reproduction degree is essential [17-19]

Among these factors we pinpointed our attention upon microelement zinc. It is a part of a variety of enzymes, has a positive effect on life duration, growth function, hypophysis, pancreatic and sexual glands, tissue repair, wound healing ability during burns, living organisms growth and reproduction. It takes an active part in metabolism (exchange of fats, proteins and hydrocarbons). It is featured by antioxidant properties, and detoxificates free radicals. Its shortage causes dwarfism, potential (libido) decrease, disorders spermiation (in case of its shortage a testosterone (which is known as "male hormone") level is getting smaller) and that in its turn causes inhibition of sexual development and decreased activity of spermatozoids for ovum fertilization [20-24]. Exactly these latter properties of zinc predetermined its selection as research subject. It is known that in the living organisms indispensable microelements predominantly perform their functions in the form of coordination (chelate) compounds. At that, the digestibility degree of microelements being in chelate form is much higher (60-70%) compared do inorganic forms (7-10%). Taking these factors into account we selected microelement zinc presented by glutaminate in chelate form as the research subject. Goal of the experiment was to study its effect on earthworm mass and degree of reproduction; as well as to establish the impact of external stress-factors, such as inundation, low quality of substrate and toxic doses of chelate zinc. An earthworm of a new brand called "Kartuliakhali (Georgian new)" that is bred in the bio-farm of Macro-Prim LLC by selectionistGuramGejadze was selected for carrying-out experiment [25].

II. COMPUTATIONAL METHOD

Based on the literature sources available for us we were unable to find any study similar to the research to be conducted, that's why we conducted an experiment according to our developed technique, and for this purpose we used the weighing method for determination of live weight of earthworms and the counting method for determination of cocoons quantity. Earthworm, cocoon, substrate and chelate zinc are among research subjects.

The following factors were studied during the test:

- ✓ Earthworms' mass change dynamics: weighing was made in three stages – on the 21st, 31st and 41st days after the start of the test (average mass change for each stage and each group in grams)
- ✓ The quantity of cocoons laid by earthworms in psc; average number of cocoons for each group in pcs in the same time interval and for each stages
- ✓ Total mass change in grams and percentage terms for control and test groups throughout the experiment and average number of cocoons in pcs and percent
- ✓ Effective and optimum dose of chelate zinc for earthworm substrate used as test specimen.

III. RESULTS AND ANALYSIS

The joint researches with participation of the Agrarian chemistry laboratory of P.Melikishvili Institute of Physical and Organic Chemistry at Iv.Javakhishvili Tbilisi State University and Bio-rational Technology Research Center focused on the study of effect of different additives and external factors on earthworms' protein mass and degree of reproduction are still in the process. Experiment was conducted according one and the same pattern by four options (Table 1).

Table 1 Pattern of conducted experiment

Options	Experiment goal	Result
I	**a)** chelate zinc effect on protein mass change and degree of cocoon reproduction	**a)** substrate saturation with chelate zinc has a positive effect on protein mass change and degree of cocoon reproduction
	b) estabishment of effective and optimum dose of chelate zinc	**b)**effective and optimum dose of chelate zinc is 0.0269g. per 300g. of substrate
II	Establishment of inundation effect on earthworms under conditions of substrate saturation with chelate zinc	Substrate saturation with chelate zinc increases earthworms' resistance to external stress factor – inundation
III	Establishment of effect of poor-quality substrate saturated with chelate zinc on earthworms	Substrate saturation with chelate zinc increases earthworms' resistance (durability) to poor-quality substrate
IV	Establishment of effect of chelate zinc toxic doses on earthworms	Earthworms can be used as biotests for toxic doses of microelements

In the first option there were made observations regarding: a) study of effect of chelate zinc added to substrate on earthworm's protein mass change and degree of reproduction and b) establishment of chelate zinc effective and optimum dose. With this end in view we tested four groups at all, each one with three repetitions. Substrate of all three repetitions of the control group was moistened with ordinary water, that of I test group – with aqueous solution containing minimal dose (0.0269g.) of chelate zinc, II test group – with aqueous solution containing normal dose (0.0539g.) of chelate zinc, and III test group – with aqueous solution containing maximal dose (0.1079g.) of chelate zinc. We selected 5 earthworms with roughly equal mass for each group repetition. First weighing and cocoon counting were made on the 21st day since the start of the experiment, while second and third ones – on 31st and 41st days, respectively. Average mass in grams, average number of cocoons in pcs were calculated for each group during all three weighing. After completion of the experiment total change of average mass in grams and in percentage terms (for all stages) for control and all three test groups was calculated, as well as average number of cocoons in pcs and in percent (Table 2).

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Table 2 Study of the chelate Zince effect on the mass change and degree of reproduction of rainworms
Test sample:Zn·Gl·3H2O

Substrate mass in container: 300g.
Quantity of rainworms in container: 5 worms

Groups (dose, g.)	Container#	Test stages												Results			
		Initial		I weighing				II weighing				III weighing				Mass change		Aver. quant. of cocoons	
		mass (g.)	mass(average)	mass (g.)	mass(average)	quantity of cocoons	quantity of cocoons(ave.)	mass (g.)	mass(average)	quantity of cocoons	quantity of cocoons(ave.)	mass (g.)	mass(average)	quantity of cocoons	quantity of cocoons(ave.)	(g.)	(%)	(piece)	(%)
Control 0.00	1	2.68	2.42	4.00	3.64	46	38	4.55	4.46	32	29.33	5.00	4.64	6	14.00	1.82	100	27.11	100
	2	1.94		4.27		31		4.33		26		4.27		14					
	3	2.65		2.65		37		4.51		30		4.64		22					
Min. 0.0269	4	2.21	2.06	4.08	4.03	34	27.33	4.46	4.51	35	33.00	4.14	3.49	28	29.33	1.95	107.14	41.45	152.9
	5	1.74		3.99		23		4.44		32		4.04		34					
	6	2.24		4.01		25		4.64		32		2.35		26					
Norm. 0.0539	7	2.16	2.33	3.98	4.40	44	39.67	3.89	4.47	36	34.67	3.41	4.05	27	29.33	1.97	108.6	34.57	127.48
	8	2.44		4.88		42		5.28		34		3.70		32					
	9	2.39		4.33		33		4.23		34		5.04		29					
Max. 0.1079	10	2.24	2.34	4.74	4.62	20	20.15	4.36	4.42	33	31.67	4.29	4.23	19	18.67	1.89	104	23.49	120.19
	11	2.18		4.31		25		4.28		35		4.26		24					
	12	2.59		4.81		16		4.63		27		4.14		13					

As is seen from the Table, in the beginning of the experiment a maximum mass gain by 1.22g. (3.64-2.42) took place at the I stage in the control group, by 0.82g. (4.46-3.64) at the II stage, and by 0.18g.(4.64-4.46) – at the III stage. In parallel with such mass change in the control group the average number of cocoons at the I-II stages was decreased first from 38 to 29.33, and then was dropped to 14.00 – at the III stage.

In the I test group, to which a minimal dose (0.0269g.) of chelate zinc was added, there was registered a mass gain by 1.97g. (4.03-2.06) at the I stage, by 0.48g. (4.51-4.03) at the II stage, while at the III stage it was decreased by 1.02g. (3.49-4.51). As for the number of cocoons (in pcs) it has been changed in the following dynamics according to stages: I – 27.33; II¬ – 67.00; III – 29.33.

In the II test group, to which a normal dose (0.0539g.) of chelate zinc was added, a mass gain equals to 2.07g. (4.40-2.33) at the I stage, by 0.07g. (4.47-4.40) at the II stage, while at the III stage it was decreased by 0.42g. (4.05-4.47). In parallel with such nature of mass change the average number of cocoons is equal to 39.67 at the I stage, 34.67 – at the II stage and 29.33 at the III stage.

In the III test group containing maximal dose of chelate zinc (0,1079g) the following dynamics of earthworms' mass change was registered: at the I stage it was increased by 2.28g. (4.62-2.34), while it was dropped by 0.2g. (4.42-4.62)

at the II stage and by 0.19g. (4.23-4.42) at the III stage. In parallel with such nature of mass change the average number of cocoons according to stages is as follows: 20.15 at the I stage, 31.67 – at the II stage and 18.67 at the III stage.

Throughout the experiment for each group and totally for all three stages an average mass gain takes place. If we conventionally take the control group index as 100% (1.82g.) then in the I test group it is 107.14% (1.95g.); in the II – 108.6% (1.97g.), and in the III test group – 104.00% (1.89g.); similarly, a total change of the degree of cocoon reproduction is of following nature: control group 100% (27.11 pieces), I test group – 152.9% (41.45 pieces), II – 127.48% (34.58 pieces) and III – 120.19% (23.49 pieces).

As is was already mentioned, the research goal also was the establishment of effect of different external stress-factors, such as inundation (second option of the experiment), poor quality (third option) and so-called toxic doses of chelate zinc (fourth option) on earthworms under conditions of substrate saturation with chelate zinc. With this end in view we set up the experiment exactly according to the above-described pattern and inundated the containers (first option). First weighing on the 21st day showed the following results: all five earthworms of the control group died, while in test group their partial death (2-4 earthworms) took place.

When conducting the same experiment under conditions of poor-quality substrate (third option) a substrate was moldy and saturated with different pathogenic organisms. The same result was obtained – all worms of the control group containers died, while in the tests group their partial death was registered.

In the fourth option of the experiment we saturated the substrates of the test groups with toxic doses of chelate zinc. When accommodating worms in the containers all earthworms of the control group were easily placed there, while in test groups, where the substrate was saturated with chelate zinc quantity four times higher compared to minimum, normal and maximum doses of the first option of main pattern, it became difficult to place earthworms in the containers, since they slid at the substrate surface and tried to run away. When inspecting the containers on the 21st day it turned out that all worms of the control group were rescued and cocoon laying was started, while not a single worm was found in the test groups' container – they either ran away or died.

IV. CONCLUSION

Based on the analysis of results obtained via conducted experiments one can draw the following conclusion: in the first experiment option the earthworm's mass gain was registered in all groups throughout the test. At that, the mass gain degree in all test groups is higher compared to control group, it reaches maximum in the II test group – 108.0%. As for the increase of cocoon reproduction degree, it is higher in all test groups compared to control one and reaches the maximum in the I test group – 152.90%. Relying on the analysis of obtained results we established an optimal, effective dose of chelate zinc – 0.0269g. per 300g. of substrate.

Thus, earthworm substrate balancing with chelate zinc has a positive effect on earthworm' mass gain and substantially improves the degree of cocoon reproduction that can be explained by biological activity and importance of microelement zinc for living organisms. We think that obtained results are of great importance for such direction of vermiculture, where protein mass is used for balancing the animal and poultry combined feed as vitamin-proteinic, high-quality concentrated additive.

Based on the carried-out experiments it was established, too that substrate saturation with chelate zinc improves earthworm's resistance (durability) to different stress-factors (such as inundation, poor-quality substrate). On the basis of experiment conducted regarding chelate zinc earthworms' toxicity it may be said that they are quite sensitive to high doses of microelements and earthworms can be successfully used as toxicity biotests that is very important due to cheapness and simplicity of this method.

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