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Researches Regarding the Use of Coacervates Essential Oils in Seed Treatment in Ecological Crop Production

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***Abstract**: Following the continuous increase of population, the request for assuring sufficient food is a fundamental objective for all farmers. In these conditions the request for ecological food production has increased as well. Answering this problem, the objective of this research paper is to establish if the microencapsulated essential oils, obtained by us in the experiments at laboratory scale, may be used to protect the wheat seed from attack of pathogens and pests in soil after seeding. The research was performed in laboratory and in experimental field. The laboratory researches was focused on microencapsulation of essential oils and on testing the phytotoxicity effect of microcapsule upon the wheat seeds and as well for test upon the population of agriotes sp, melolontha melolontha larvae stage I, II. The microcapsules were obtained by a process of complex coacervation, and they have a central core formed by an essential oil, covered with a shell made of crosslinked hydrolyzed collagen. In the experimental field was evaluated the intensity of pests attack frequency. The results of phytotoxicity tests show that all the products used in seed treatments don't have any phytotoxic influence at the used doses; on the contrary we identified a stimulating effect upon the plant growth. The researches regarding the influence upon the melolontha melolontha populations show the products with essential oil from Thymus vulgaris, Satureja hortensis, Ocimum basilicum decreasing the intensity of attack by comparing with control variant without any treatment. By comparing with control treated using imidacloprid, in laboratory, the efficacy of treatment was around 50% from that one. In the experimental field we found the decreasing significantly of aphidae attack frequency. Analyzing the results obtained in this research we can said: "all the seed treatments based on own microencapsulated essential oils, can be used successfully for wheat pest protect in ecological system".*

***Keywords:** microencapsulated essential oil, biopesticides, collagen hydrolysates, seeds treatment, biostimulant*

1.Introduction

At global level the dynamics of the population records ascending trend that shall manifest in an increase of the request for food and thus the farmers and agricultural experts will have to develop new technologies which shall ensure a sustainable and stable growth of the productions without being affected by the environmental factors.

Wheat crops have decreased at global level from 227 mil ha in 1997 to 218 mil ha in 2017, while in Romania the wheat crops have decreased from 2.38 mil ha in 1997 to 2.05 mil ha in 2017, whereas average productions increased continuously from 3010 kg/ha in 1997 to 4888 kg/ha in 2017 [1].

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Within this context, there is a growing accent on increasing the quality of the seed which will represent a guarantee increased productions. Beside the conditions regarding the quality of the seeding material, a high importance should be granted to protection of seeds after sowing [2, 3], and to the possibility of applying biostimulating substances which shall determine an increase of the germinating energy, simultaneous emergence [4].

Within leather industry, the efficiency of transforming rough skins into finite product is of 20-50%, the difference being represented by a series of waste: wool, fat, blood, proteins. Following the processing, from the waste are extracted amino-acids with different practical usage [5]. In the dedicated literature are presented various technological solutions of recovering waste obtained by processing rough skins: hydrolyzed collagen, pigments, surfactants. By using enzyme chemical processes are obtained extracts of long-chain polypeptides, which can be used in seed treatments [6, 7]. To these technological solutions of obtaining products with bio-active effect, are added also the posibility of micro-encapsulating substances with pesticide effect – which released slowly decrease the amounts of pesticides used and increase the period of seed protection [8].

Many researchers and patents presented a series of technical solutions of obtaining micro-capsules [9-11]. A classification of materials used in producing microcapsules according to the biodegradable rate was performed [12]: a) amidon and amylose based system; b) other polysaccharides (cellulose and derivatives, chitin, chitosan, dextran, alginate); c) proteins (casein, albumen, gelatin); d) lipophilic materials (rubber and wax); e) synthetic polymers (polyvinyl alcohol, polylactate, polyglycolate, polyesters, polyamines, polyamides, polyacrylamide); f) other (polyhydroxybutyrate, tannins, polyhydroxyvalerate) lignins, resins and biopolymers modified by substitution"veining" or "grafting".

In this sense, the researches from the last couple of years focused on encapsulating volatile oils which are biodegradable, have specific actions against some insects or fungus, aren not resistant [13, 14].

Were tested essences of: *Lavandula angustifolia*, Cedrus spp. and species of Pepper for pest control being called "green pesticides" [15] .

Within this paper are presented the rezults of the tests regarding the action of micro-capsules on volatile oil basis suspended in a mixture of amino-acids obtained through enzyme hydrolise of subproducts resulted by primary processing of bovine skin, against wheat crops, after seed treatment and before sowing.

2.Materials and methods

The bovine leather by-products are obtained from SC Pielorex SA, Romania for collagen extraction. Hydrated calcium oxide p.a. from SC Cristal R Chim SRL for alkaline hydrolysis. Glacial acetic acid p.a (SC Cristal R Chim SRL) for pH corrections. Alcalase 2.5 L from Novozymes, Denmark, with activity of 2.5 enzymatic units/g, optimal enzymatic activity at temperature = 60 ºC and *p*H range = 7-9. Essential oils were purchased from Naturela, Glutaraldehyde 50% from Scharlab SL Spain, Romania. Sodium carboxymethyl cellulose salt from Sigma-Aldrich**,** Germany.

The objective of the researches was to evaluate the action of products based on microcapsules with essence oils suspended within an extract of amino-acids (MEO) on wheat crop pests.

The amino-acids extract as collagen hydrolysates for essence oils coacervations are obtained from bovine hide shavings by chemical-enzymatic hydrolysis at atmospheric pressure and temperature of 60-80°C for 6-8 h, in vessel equipped with thermostated heating system, stirring system, refrigerent for vapours. The collagen hydrolysates were collected after vacuum filtration by Nuce filter.

The collagen hydrolysates were analysed in terms of dry substance (SR EN ISO 4684:2006) and total ash (SR EN ISO 4047:2002) by gravimetric methods, total nitrogen and protein substance (SR ISO 5397:1996), aminic nitrogen (ICPI protocol) by volumetric methods, *p*H (SR EN ISO 4045:2008) by potentiometric method.

The amino acid composition of collagen hydrolysates were determined by high-performance liquid chromatography, according to SR EN ISO 13903 by HPLC Thermo Electron, Finningen Surveier cu Diode Array Detector and gas chromatography by AGILENT 7000 GC/MS TRIPLE QUAD Gas Chromatograph.

Dynamic Light Scattering (DLS) investigation of collagen hydrolysates was performed using the ZetaSizer device Nano ZS from Malvern, UK, to determine particle size and distribution.

Microencapsulation process of essential oils was carried out by complex coacervation with gelatine as shell material. Thus, 3% of hydrolyzed collagen solution was taken into a reaction vessel and temperature was raised to 50ºC and essential oil was added under high stirring at 1000 rpm to form an emulsion. Coacervation was performed by gradual addition of aqueous anionic polymer, sodium carboxymethyl cellulose salt (CMC-Na) of 1.5% concentration for 30 min, under high agitation. *p*H value of the mixture was adjusted to 3.5 with solution 5% acetic acid. Temperature of the vessel was reduced to about 5ºC in 30 min, keeping a high stirring. The crosslinking of the polymer microcapsules was performed by addition of 50% glutaraldehyde solution, at glutaraldehyde / polymer ratio of 4-6 mmol/g. The suspension was stirred for 10 h, the temperature raising from 5 to 25ºC. *p*H value of mixture was adjusted to 8 by adding sodium bicarbonate solution, and stirring was maintained until 5 h. The hardened microcapsules were filtered, rinsed with cold water and methanol, and finally were vacuum dried.

In order to achieve the researches'objectives, were chosen two directions regarding the treatment of seeds with these products: 1. testing phytotoxicity of products on seed germination and growth of wheat plants; 2. Testing the insecticide effect of these products.

The testings started with the treatment of 10 kg of Arnold wheat grains, MMB 44.6, MH 76.1 for each experimental version. The experimental versions were presented in table 1.

Table 1
Experimental version of the wheat grains treatment

V1	Ceck untreated	Mt
V2	Gaucho 600FS, 600g/l imidacloprid, 8 L/t	Gaucho 600FS
V3	Amino acids complex as hydrolysate collagen	AEHC
V4	Amino acids complex as hydrolysate collagen with microcapsule Thymus vulgaris 15%	AEHCTV15
V5	Amino acids complex as hydrolysate collagen with microcapsule Thymus vulgaris 30%	AEHC TV30
V6	Amino acids complex as hydrolysate collagen with microcapsule Satureja hortensis 15%	AEHC SH15
V7	Amino acids complex as hydrolysate collagen with microcapsule Satureja hortensis 30%	AEHC SH30
V8	Amino acids complex as hydrolysate collagen with microcapsule Ocimum basilicum 15%	AEHC OB15
V9	Amino acids complex as hydrolysate collagen with microcapsule Ocimum basilicum 30%	AEHC OB30

Testing phytotoxicity was performed within the lab in a monofactorial experience with the versions previously presented. Testing the germination after having applied the treatments, was performed according to standard SR1634/1999 on Determing the germination of seeds and ISTA Handbook on Seed Evaluation. ISTA rules 2013. There were 4 rehearsals of 100 seeds for each version. The testing

method was TP (top paper), in roll. The samples were incubated at 20^{o}C temperature and 80% humidity, in a Caloris ITU 150 incubator. The evaluation of germination was performed at and 8 days.

Testing the insecticide effect of microcapsules with essence oils suspended in a colagen extract, was performed in 3 experiences in the laboratory.

The laboratory experiments were performed in vegetation pots of 25x25x13 cm size. The soil used was priorly strained, in order to remove possible pests present in the soil. Each pot was filled with soil on a height of 10 cm, and afterwards 100 wheat grains were homogeneously distributed, with the help of a template with 2.5x2.5 cm distance. Pests were added according to the experimental protocol and another 2 cm soil were added above the grains.

The experiences were of monofactorial type on the previously presented 9 versions of treatment, in 10 rehearsals (5 rehearsals were removed after the plants emergence, in order to evaluate the frequency of the attacks on germinated grains and plants). After preparing the vegetation pots, they were watered up to the water capacity in the field. Evaluating the attack frequency at the emergence moment was performed on 5 vegetation pots (rehearsals) out of the 10 put for each version. Each vegetation pot was emptied, soil was removed, the non-germinated seeds, full plants and attacked plants were counted.

-Evaluating the influence of treatments with MEO on wire worm (agriotes sp)

In each pot, after the homogeneous distribution of seeds, were added 2 larvae of *Agriotes* spp. of age II , above which were added 2 cm of soil, to observe: frequency of the attack on germinated seeds and on plants up to stage BCCH 14-15 (3 weeks after emergence); survival of plants.

In each pot were introduced, in soil, a larva of *Agriotes spp*. age II, to observe: frequency of attack on germinated seeds and of plants up to stage BCCH 14-15 (3 weeks after emergence); survival of plants.

At the emergence were used 5 pots, from which soil was removed and was determined the percentage of germinated seeds, attacked and unattacked seeds.

-Evaluating the influence of treatments with MEO on white worms (Melolonta melolonta)

In each vegetation pot were introduced, in the soil, 2 larvae of *Melolonta melolonta* age II, to observe, s-a urmărit: frequency of the attack on the germinated seed and plants up to the stage BCCH 14-15 (3 weeks after emergence); survival of plants.

-Evaluating influence of treatments with MEO on larvae afidae

To perform the experiment were used 5 vegetation pots each of 5 plants of wheat / pot for each version. After emergence, within stage BBCH 12-13 (2-3 leaves completely formed), on each plant were put 5 larvae of aphidae, observing after 3 weeks the number of aphidae on each plant.

-Testing the effect of MEO insecticide on the field was performed by two experiences

Performing the experiments on the field started with probing regarding the identification of the area within the experiment field, with increased incidence of the pests. After the probing the density of pests within the chosen field was of 2-3 wire worms /sq 3-4 white worms /sq m. No aphidae were identified before sowing/planting.

Each experimental version was planted in 4 rehearsals of 100 square metres, seeding norm of 300 germinated grains/sqm.

The number of plants/ m^2 at emergence and at 3 weeks after emergence was determined for each experimental version and through reports at untreated witness value to calculate the intensity of the attack.

3.Results and discussions

The composition of amino nitrogen (using the Sörensen method) indicates an average molecular weight of about 7200 Daltons for collagen hydrolysate extracted from bovine leather by-products. The composition of amino acids in collagen hydrolysates is an important feature, because they are plant grow stimulators.

The amino acids composition of collagen hydrolysates, determined by chromatography (HPLC), is presented in Figure 1.

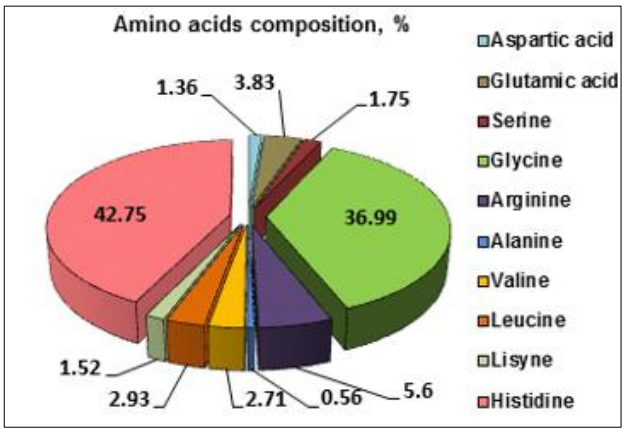

Figure 1. Amino acids composition in collagen hydrolysate

The distribution of particle populations determinated by DLS, presented in Figure 2 shows there is a minor fraction (1.90%) in the range of 1000-10000 nm, an intermediate fraction (22.20%) in the range of 1-10 nm and a majority (75.90%) in the range of 100-1000 nm.

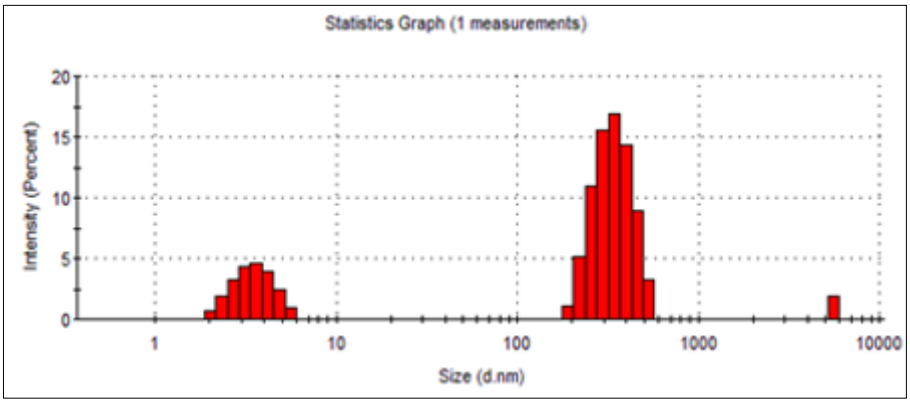

Figure 2. Particle size and distribution in collagen hydrolysate

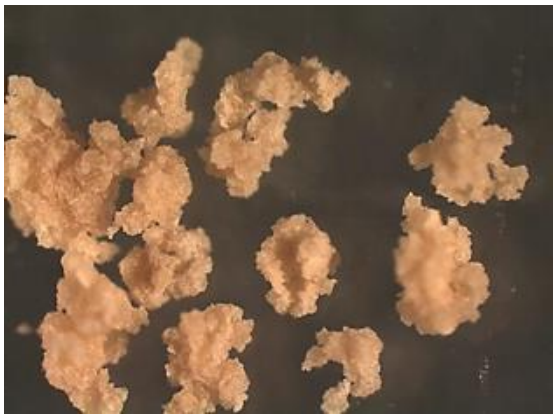

Figure 3. Optical microscopy of microcapsules with experimental essential oils

The results of encapsulation experiments of essential oils in collagen hydrolysate recovered from leather industry are synthetically presented in, Figure 3 optical microscopy and figure 4 SEM.

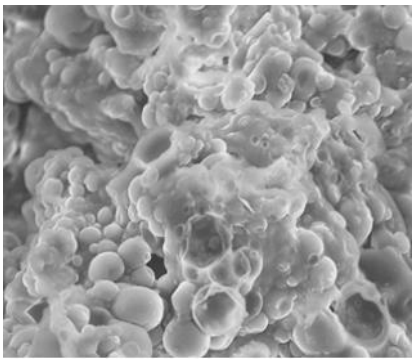

Figure 4. SEM of microcapsules with experimental essential oils

Microcapsule agglomerates, visible at 20X magnification, are due to the high molecular weight polypeptides from the residue of collagen hydrolysates in the reaction mass, which act as a binder of the microcapsules formed by coacervation.

The visible cracks and pores may be caused by the water loss from the collagen hydrolysate network during the sample preparation for SEM.

It was proved that the collagen hydrolysate extracted from solid wastes from tanneries can be successfully used in microencapsulating essential oils.

Testing phyto toxicity

Analysing the results of the rearches regarding the influence of tratments after 4 days of incubation, can be observed that the percentage of germinated grains was of 83.75% at Ceck, 82.50% at treatment with Gaucho 600FS and varied between 87 and 88.75% at treatments with MEO. Reported to Ceck, the treatment with Gaucho a determined an unsignificant decrease of germination, and applying MEO determined significant increases of germination between 4 and 4.5%.

At 8 day scan be observed that the geminated seeds percentage varied between 93.25% in the case of ceck without treatment and above 98.25% in the case of MEO treatment, and the increases compared to ceck are significant. Treatment with Gaucho determined an unsignificant decrease of the germinated grains percentage.

Table 2 show the influence of microencapsulated essential oil suspended in amino acids complex as hydrolysate collagen used for treated the wheat grains upon the germination, in laborator condition.

Table 2. Germination of the wheat grains treated with MEO suspended in amino acids complex as hydrolysate collagen

Versions		% germination 4 days			% germination 8 days		
		% germinated grains	dif	Number of abnormal germs	% germinated grains	Dif	Number of abnormal germs
V1	Ceck	83.75	Mt	2	93.25	Mt	2
V2	Gaucho 600FS	82.50	-1.25	3	91.75	-1.50	3
V3	AEHC	87.75 *	4.00	1	98.25 *	5.00	1
V4	AEHC TV15	87.75 *	4.00	1	98.50 *	5.25	1

V5	AEHC TV30	88.00 *	4.25	1	98.50 *	5.25	1
V6	AEHC SH15	88.00 *	4.25	1	98.00 *	4.75	1
V7	AEHC SH30	88.25 *	4.50	1	98.75 *	5.50	1
V8	AEHC OB15	88.25 *	4.50	1	98.50 *	5.25	1
V9	AEHC OB30	88.00 ·	4.25	2	98.50 *	5.25	1
		LSD 5%	3.89			4.11	
		LSD 1%	6.38			7.20	
		LSD 0.1%	9.91			10.65	

Evaluating the influence of treatments based on microcapsules with essence oils suspended in a mixture of amino-acids on pests, in laboratory conditions

Evaluating the influence of treatments based on MEO against wire worm (agriotes sp), in laboratory conditions

In each vegetation pot were introduced, in the soil, a larva *Agriotes* spp. of age II, observing: frequency of attack on germinated seeds and on plants up to stage BCCH 14-15 (3 weeks after emergence); survival of plants. At plants'emergence were used 5 pots, for which was determined the percentage of pest attacked germinated grains. All the calculations regarding the percentage of attacked plants was reported only to germinated grains.

In table 3 are presented the results of determing the influence of treatments with MEO applied to wheat grains, on agriotes spp. pest. Analysing the data presented in the table it can be observed that at emergence the percentage of attacked plants varied between 55.2% at untreated witnes and 86.4% at witnes treated with Gaucho 600, at other versions treated with MEO the percentage of unattacked plants varied between 58.4 and 61.2%. Reported to the untreated witnes , al the versions to which were applied treatments with MEO presented statistically ensured increases of the percentage of unattacked plants at emergence. The increases were comprised between 3.2% and 6% at treatments with MEO and 31.2% at treatment with Gaucho 600.

Table 3. The influence of treatments applied on the percentage of wire worm unattacked plants (*agriotes spp*) in laboratory conditions

Versions		At emergence		At 3 weeks after emergence	
		BBCH 09	Dif	BBCH 15	Dif
V1	Ceck	55.2	Mt	35.0	Mt
V2	Gaucho 600FS	86.4	31.2 ***	88.0	53.0 ***
V3	AEHC	58.4	3.2	38.4	3.4 *
V4	AEHCTV15	59.6	4.4 *	41.8	6.8 ***
V5	AEHC TV30	61.2	6.0 **	43.6	8.6 ***
V6	AEHC SH15	59.8	4.6 *	42.4	7.4 ***
V7	AEHC SH30	61.2	6.0 **	44.4	9.4 ***
V8	AEHC OB15	59.0	3.8	40.8	5.8 ***
V9	AEHC OB30	60.0	4.8 *	41.8	6.8 ***
		LSD 5%	3.33		2.37
		LSD 1%	5.14		3.84
		LSD 0.1 %	6.93		5.30

At 3 weeks after the emergence the percentage of unattacked plants varied between 88% at treatments with Gaucho and 35% at untreated witnes, at the rest of versions treated with MEO varied between 38.4% and 44.6%. Compared to the witnes untreated, all other versions presented statistically ensured increases of the unattacked plants number.

Evaluating the influence of treatments based on microcapsules with essence oils suspended in a mixture of amino-acids upon white worms (Melolonta melolonta)

In Table 4 are presented the results of determing the influence of treatments with MEO applied to wheat grains, upon *Melolonta melolonta* pest.

From the analyses of data can be observed that at emergence, the percentage of plants attacked varied between 66.8% at untreated witnes and 86.4% at witnes treated with Gaucho 600, at the rest of versions treated with MEO the percentage on unattacked plants varied between 70.6 and 75.8%. Reported to the untrated witnes all the versions to which were applied treatments with MEO presented statiscally ensured increases of the unattacked plants at emergence. The increases were comprised between 3.2% and 6% at treatments with MEO and 31.2% at treatment with Gaucho 600.

At 3 weeks after emergence, the percentage of plants unattacked varied betwween 89.4% in treatment with Gaucho and 35% at untreated witnes,at the rest of versions treated with MEO varied between 38.4% and 44.6%. Compared with the untreated witnes, all the other versions treated with MEO presented statistically ensured increases of the unattacked plants number. Comprised between 4% and 9.6%.

Table 4. The influence of treatments with MEO applied upon the percentage of plants unattacked by *Melolonta melolonta* in laboratory conditions

Versions		At emergence		la 3 weeks after emergence	
		BBCH 09	Dif	BBCH 15	Dif
V1	Ceck	66.8	Mt	64.6	mt
V2	Gaucho 600FS	86.4	19.6 ***	89.4	24.4 ***
V3	AEHC	70.6	3.8 *	67.8	3.2
V4	AEHCTV15	74.0	7.2 **	71.8	7.2 **
V5	AEHC TV30	75.8	9.0 ***	74.2	9.6 ***
V6	AEHC SH15	72.2	5.4 *	72.0	7.4 **
V7	AEHC SH30	73.8	7.0 **	72.8	8.2 ***
V8	AEHC OB15	70.8	4.0 *	68.6	4.0
V9	AEHC OB30	71.8	5.0 *	69.6	5.0
		LSD 5%	3.68		3.61
		LSD 1%	5.89		5.78
		LSD 0.1 %	8.09		7.95

Evaluating the influence of treatments based on microcapsules with essence oils suspended in a mixture of aminoacids on aphidae

Table 5. The influence of treatments applied on the percentage of plants attacked by aphidae in laboratory conditions

Versions		at 3 weeks after emergence	
		BBCH 15	Dif
V1	Ceck	66.4	Mt

V2	Gaucho 600FS	88.2	21.8 ***
V3	AEHC	68.8	2.4
V4	AEHCTV15	72.4	6.0 **
V5	AEHC TV30	75.2	8.8 ***
V6	AEHC SH15	73.8	7.4 ***
V7	AEHC SH30	73.6	7.2 ***
V8	AEHC OB15	68.8	2.4
V9	AEHC OB30	70.4	4.0 *
		LSD 5%	3.86
		LSD 1%	5.96
		LSD 0.1 %	8.04

Regarding the attack of aphidae on wheat plants after emergence, can be observed that the number of plants attacked was 66.4% in ceck and 88.2% in case of versions treated with Gaucho. At versions to which were applied treatments with MEO, the percentage of attacke plants varied between 68.0% and 75.3%.

Compared to ceck, treatment with Gaucho recorded an increase of 21.8% in the percentage of unattacked plants. Treatments with AEHC TV30, AEHC SH15, AEHC SH30 determined very significant increases of the number of unattacked plants between 7.2% and 8.8%. In the case o treatments with AEHC and AEHC OB15 the increase in the percentage of unattacked plants is not statistacally ensured.

Evaluating the influence of treatments based on microcapsules with MEO upon pests, in field conditions.

Testing the insecticide effect of microcapsules with MEO was performed during experiments on field (Wire worms)

In Table 6 are presented results from the on field testing regarding the influence of treatments with MEO upon the attack of wire worms (*agriotes spp*).

Table 6. The influence of treatments with MEO upon the percentage of unattacked plants by wire worms (*agriotes* spp) in field conditions

Version		At emergence		at 3 weeks after emergence	
		BBCH 09	Dif	BBCH 15	Dif
V1	Ceck	60,2	Mt	47.8	Mt
V2	Gaucho 600FS	94.0	33.8 ***	93.8	46.0
V3	AEHC	62.4	2.2	49.8	2.0
V4	AEHCTV15	64.2	4.2 *	50.6	2.8 *
V5	AEHC TV30	65.8	5.6 **	51.2	3.4 *
V6	AEHC SH15	63.6	3.4 *	49.8	2.0
V7	AEHC SH30	64.8	4.6 **	50.2	2.4
V8	AEHC OB15	62.6	2.4	50.0	2.2
V9	AEHC OB30	63.8	3.6 *	50.4	2.6
		LSD 5%	3.35		2.75
		LSD 1%	5.35		4.40
		LSD 0.1 %	7.36		6.05

Analysing the data presented, at the emergence it can be observed that the percentage of unattached plants increased significantly for SCTV15, AEHC TV30, AEHC SH15, AEHC SH30, AEHC OB30. At the 15% concentration microcapsule with volatile oil, only in the case of treatment with Thymus vulgaris, Satureja hortensis was significant.

At 3 weeks after the emergence, the percentage of plants attacked was between 47.8% at untreated ceck and 93.8% at treatment with Gaucho 600FS, and at versions treated with MEO was between 49.8% and 51.2%. It can be observed that the increase of the percentage of unattacked plants has significatly increased at treatment with AEHCTV15 and AEHC TV30.

Testing the insecticide effect of microcapsule with MEO was performed in on field experiments, white worms (melolonta melolonta)

In table 7 were presented the results of determing the influence of treatments with MEO based products applied to wheat grains, upon/against melolonta melolonta(white worms) pest.

Table 7. The influence of treatments applied upon the percentange of unattacked plants

Version		At emergence			At 3 weeks after emergence		
		BBCH 09	Dif		BBCH 15	Dif	
V1	Ceck	7.6	Mt		71.4	Mt	
V2	Gaucho 600FS	96.2	21.6	***	91.8	20.4	***
V3	AEHC	75.0	0.4		71.8	0.4	
V4	AEHCTV15	78.8	4.2	*	75.2	3.8	*
V5	AEHC TV30	81.0	6.4	**	76.6	5.2	*
V6	AEHC SH15	76.4	1.8		73.6	2.2	
V7	AEHC SH30	78.7	4.1	*	74.8	3.4	
V8	AEHC OB15	75.2	0.6		72.6	1.2	
V9	AEHC OB30	76.4	1.8		72.4	1.0	
		LSD 5%	3.96			3.77	
		LSD 1%	6.33			6.03	
		LSD 0.1 %	8.70			8.29	

Analysing the data presented in the table can be observed that at emergence the percentage of attacked plants varied between 74.6 % at untreated ceck and 96.2% at treated witnes with Gaucho 600 at 81.0%.

Compared to untreated ceck treatments with AEHCTV15, AEHC TV30 and AEHC SH30 were determine significant increases of unattacked plants percentage.

At 3 weeks after emergence the oercentage of unattacked plants varied between 91.8% at treatmentwith Gaucho600FS and 71.4% at untreated ceck, at the rest of versions treated with MEO based products varied between 71.8% and 76.6%. Compared to untreated ceck all the other versions treated with MEO products presented increases in the percentage of unattacked plants, the increases being significant only in the cases of versions AEHCTV15, AEHCTV30.

Following the researches were observed among the 3 types of MEO based products increases of unattacked plants percentange, in laboratory and field conditions, determing only by those on base of Thimus Vulgaris and partially with Satureja hortensis essence oils. The results obtained are only in accordance with the results obtained by Nielsen (2005).

4.Conclusions

Treatment with MEO based products has not determined phytotoxic effects.

All MEO based products presented a stimulating effect on germination.

In laboratory, under the influence of treatments with MEO based products has increased the percentage of plants unattacked both at emergence as well as at 3 weeks after the emergence, for all 3 pests studied.

In field conditions, under the influence of treatments with MEO based products, has increased the percentage of unattacked plants.

In field, the percentage of unattacked plants percentage has significatly increased at treatment with AEHCTV15, AEHC TV30 and AEHC SH30 for *agriotes spp* and AEHCTV15, AEHC TV30 fors *melolonta melolonta.*

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References

1.***http://faostat.fao.org/site/567/DesktopDefault.aspx?PageID=567#ancor
2.BAZZONI, E., SANNA-PASSINO, G., MORETTI, M. D. L., Recent Progress in Medicinal Plants, Vol. 8, Phytochemistry and Pharmacology II. Houston, TX: Sci Tech Publishing LLC, 2002, p. 313.
3.SINGH, D., Advances in plant biopesticides, New Delhi, Springer, ISBN 978-81-322-2006-0, eBook, 2014.
4.DRAGICEVIC, V., GOSIC-DONDO, S., JUG, I.; SRDIC, J.; SREDOJEVIC, S. The Proceedings of the 46th Croatian and 6th International Symposium on Agriculture, Opatija, Croatia, Pospisil, M., Ed., Izdava: University of Zagreb, Croatia, 2011, p. 654.
5.NICULESCU, M., BAJENARU, S.; GAIDAU, C., SIMION, D., FILIPESCU, L., Rev. Chim., **60**, (10), 2009, 1070
6.KOLOMAZNIK, K., ADAMEK, M., ANDEL, I., UHLIROVA, M., J. Hazard. Mater., 160, no. 2-3, 2008, p. 514.
7.SCHMIDT, M. M., DORNELLES, R. C. P., MELLO, R. O., ET AL., J. Appl. Pharm. Sci., 5, no. 3, 2015, p. 123.
8.NICULESCU, M.-D., EPURE, D.-G., LASON – RYDEL, M., GAIDAU, C., GIDEA, M., ENASCUTA, C., EuroBiotech J., 3, no. 3, 2019, p. 160.
9.OCAK, B., ASLAN, A., GULUMSER, G., J.A.L.C.A., 106, 2011, p. 232.
10.SLIWKA, W. Angew. Chem. Int. Ed. Engl., 14, 1975, p. 539.
11. YAN, N. U.S. Patent, 6.974.592, 2005.
12.WILKINS, R. M., Taylor and Francis Ltd., Ed., London, U.K., 1990, p. 325.
13.SPARKS, R. E., JACOBS, I. C., SCHER, H. B., Ed., Marcel Dekker, Inc: New York, 1999, p. 3.
14.SCHAAFSMA, A.W., TAMBURIC-ILINCIC, L., Plant Dis., 89, no. 10, 2005, p. 1109.
15.KOUL, O., WALIA, S., DHALI WAL, G. S., Biopestic. Int., 4, no. 1, 2008, p. 63.

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