Qualitative and chemical evaluation of nano clove extract and study of its effect on the quality of minced local chicken meat stored for different periods

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Abstract

This study aimed to evaluate the chemical evaluation of local minced chicken meat preserved in polyethylene casings treated with nano clove extract. The study included six treatments with different concentrations added to polyethylene bags: the first treatment (A) as a control treatment (without addition), the second treatment (B) nano-zinc at a concentration of 20 mg/ml, and the third treatment (B1) clove alcohol extract at a concentration of 100 mg/ml.The fourth treatment (B2) clove alcohol extract 125 mg / ml, the fifth treatment (B3) nano-zinc (20 mg / ml) with the lowest concentration of clove alcohol extract (100 mg / ml) at a ratio (1-1), the sixth treatment (B4) zinc Nano (20 mg/ml) with the highest concentration of clove alcohol extract (125 mg/ml) at a ratio (1-1), minced chicken meat was kept in these treated bags separately and stored for periods of (10,5,0) days by refrigerationand some chemical tests were done for her, and the results of this study were as follows:

1- The sixth treatment (B4) showed a significant increase (P<0.05) in the percentages of both moisture and protein of chicken meat during the different cold storage periods compared with the control treatment.

2- The results of the different addition treatments showed a significant decrease (P<0.05) on the percentage of fat and ash.

هدفت هذه الدراسة الى التقييم الكيميائي للحم الدجاج المحلي المفروم والمحفوظ بأغلفة البولي اثيلين المعاملة بمستخلص القرنفل النانوي, وتضمنت التجربة حفظ لحم الدجاج المفروم بالتبريد بدرجة حرارة 4م لمدة 10 ايام

وشملت الدراسة ست معاملات بأضافة تراكيز مختلفة عوملت اكياس البولي اثيلين بها: المعاملة الاولى (A) معاملة السيطرة (بدون أضافة), والمعاملة الثانية (B) النانو زنك بتركيز 20 ملغم/مل, المعاملة الثالثة (1B) مستخلص القرنفل الكحولي بتركيز 100 ملغم /مل المعاملة الرابعة (2B) مستخلص القرنفل الكحولي 125 ملغم /مل, المعاملة الخامسة (3B) الزنك النانوي (20ملغم/مل) مع التركيز الاقل من مستخلص القرنفل الكحولي (100ملغم / مل) بنسبة (1-1 (, المعاملة السادسة(3B) الزنك النانوي (20ملغم/مل) مع التركيز وخزنت بمدد (10,50) يوماً بالتبريد, واجريت لها بعض الاختبارات الكيميائية, وكانت نتائج هذه الدراسة على النحولي :

- 1- اظهرُتُ المعاملة السادسة (4B) ارتفاعاً معنوياً (P<0,05) في نسبُ كلّ من الرطوبة والبروتين للحم الدجاج خلال مدد الخزن المختلفة بالتبريد مقارنة مع معاملة السيطرة.
 - 2- بينت نتائج معاملات الاضافة المختلفة وجود انخفاض معنوي (P<0,05) على نسبة الدهن و الرماد .</p>

INTRODUCTION:

Poultry meat and its products have a high nutritional value compared to other types of meat, and constitute a major food source of essential amino acids in human nutrition and according to the chemical nature of chicken meat, it is subject to spoilage as a result of oxidation of fat, which is one of the most factors affecting the quality of food, as oxidation negatively affects the taste and flavor, which reduces the storage life of meat and its products. Therefore, the use of chemical and industrial materials to preserve meat and meat products has become essential to avoid rapid spoilage of products, but some of them have a bad impact on human health (2). The continuous increase in demand for meat products and the increasing competition and health concerns have led the meat industry to adopt new and innovative methods. Therefore, the use of techniques such as nanotechnology can have a significant impact in the meat industry by using it to improve the quality characteristics, as well as to accurately deliver biologically active substances to the target (3).It can have a significant impact in the process of prolonging the period of food preservation, improving quality and safety characteristics, and reducing the percentage of fat and salt, in addition to its applications in packaging materials (3)The use of alternative and safe materials, such as food additives of vegetable origin, which have the ability to prevent contamination of food products, knowing that these materials do not cause any harm to the nature of the product or to the health of the consumer (4).On the other hand, clove buds are characterized by their effectiveness in extending the duration of food preservation due to the presence of many effective compounds in them, and the possibility of benefiting from these materials in the medical, food and industrial fields (5). In view of the scarcity of applied studies in this regard in Iraq, this study aimed to:

1- Detection of the active substances of clove buds.

2- Studying the effect of polyethylene casings treated with nano-extract on the qualitative traits of minced chicken meat and cold-preserved at 4° C for the periods (0, 5, 10).

Materials and methods:

1- Preparation of alcoholic clove extract:

The clove buds that we got from the local markets in Tikrit were collected, classified by experts in the Department of Horticulture / College of Agriculture / Tikrit University, crushed and preserved in tight plastic containers at laboratory temperature until use.

1-2 alcohol extraction method:

50 g of clove powder was taken in the extraction unit (Soxhelt) and 350 ml of ethyl alcohol was added to it at a concentration of 85%, and the extraction process continued for 12 hours at a temperature of 40 degrees Celsius in the extraction unit, and then using the Rotary Evaporator Vacuum at (35) degrees Celsius) 6). Where we obtained a dense extract, the drying was completed using a convection oven at a temperature not exceeding (40) degrees Celsius, and then the final extract was saved after drying until use.

2- Preparation of solutions of nanomaterials and plant extracts:

The solutions were prepared from the nanomaterials and the plant extract as basic solutions used to prepare the necessary dilutions from them, as shown in Table (1):

2-1- Nano zinc: ZnNPs:

Prepared nanozinc were obtained and the size of the nanoparticles was 30-60 mn from American origin. The concentrations of nanozinc (ZnNPs) were prepared by dissolving 20 mg of the base material in 10 ml of hot distilled water (7).

2-2 Clove alcoholic solution:

Prepare a solution of clove extract by dissolving 1-1.25 g of the extract in 10 ml of hot distilled water (7).

2-3 nano zinc solution with clove alcohol extract:

The solution was prepared by dissolving 20 mg of ZN-NPs with 100 mg (lower concentration) of clove alcohol extract, and the same concentration of 20 ZN-NPs/mg with 125 mg (higher concentration) of clove alcohol extract in 10 ml of hot distilled water in a ratio of 1: 1 for the two concentrations (7).

Table (1) Treatments symbol and concentrations of materials used in the experiment

Treatments symbol	Treatments
А	Control treatment without adding
В	Nano zinc at an concentration of 20 mg / ml
B1	Alcoholic clove extract at an concentration of 100 mg/ml
B2	Alcoholic clove extract at an concentration of 125mg/ml
В3	Clove Extract, Nano, Lowest concentration (1:1)
B4	Nano Clove Extract Highest concentration (1:1)

Preparing the storage bags:

The sealable polyethylene casings intended for preserving meat, bearing the trade name (falcon), of Emirati origin, obtained from the local markets in the city of Tikrit, were prepared and treated by contaminating them with a sterilized brush with different concentrations of extracts separately, minced chicken meat was preserved inside it only The following sequential steps:

1- 1200 g of skinless local chicken meat was taken, then it was minced with an electric chopper.

2- The polyethylene bags were prepared and treated with a sterile brush with the previously mentioned treatments.

3- Put 200 g of minced meat in the treated bags.

4- Chemical and qualitative tests were conducted on day (0).

5- Meat bags were kept refrigerated at 4°C.

6- Repeat the chemical and qualitative tests on the 10th and 5th day of preservation, respectively.

7- Re-tested, chemical and qualitative on the (10) day of preservation.

4- Qualitative chemical detection of some effective groups of alcoholic clove extract:

Phenols were detected (8), while resins, saponins and tannins were detected according to the method (9), and flavones according to (10),

Table (2): Detect	tion methods for the active sub	stances of clove alcoholic extract
Effective compound	detection index	Detector used
phenols	bluish green color	iron chloride 1%
resins	Orange to yellow	Ethyl alcohol in boiled distilled water + HCL
soaps	Long lasting thick foam	A- Shake the extract
tatinas	A white precipitate appears	b- mercuric chloride
flavonoids	white gelatinous precipitate	a- lead acetate 1%
Glycosides	A bluish-green color appears	B - ferric chloride 1%
Alkaloids	vellow color	Ethyl alcohol NaOH

as for glycosides, they were detected according to the method of Al-Sheikhly and his group (11), and finally alkaloids according to (12).

Chemical tests:

5-1 Moisture determination

The moisture content of minced chicken meat was measured using the methods mentioned in AOAC (13) on the basis of weight loss of samples before drying.

Then, using a weight of 5 g, it was placed in an eyelid with a known weight in advance and dried in an electric oven at a temperature of 105 ° C until the weight was stable and with three replications, the moisture percentage was calculated according to the following equation:

Moisture percentage %=<u>Weight before drying</u> - weight after drying original weight 100

5.2 Protein determination ::

The percentage of crude protein in minced chicken meat samples was estimated based on the A.OAC method (13) and using the Micro kildah device by taking 0.2 g of minced chicken meat and for each treatment placed in the digestion tube of the device and adding 5 ml of concentrated sulfuric acid (95%) and two drops of sulfuric acid (95%) Perchloric (HCLO4) The mixture was left for 24 hours and heating was used to speed up the digestion process, as the color changed from black to yellow, then to transparent, then distilled by adding 10 ml of NaOH (0.1 N) and the released ammonia was collected in a 50 ml flask containing 25 ml of acid Boric 2% with two drops of Bromocresol green and Methyl red evidence. It was rubbed with hydrochloric acid HCL at a concentration of 0.05 N until its color changed from green to red. The protein percentage was calculated according to the following equation:

Protein	percentage
$\eta_0 = \frac{HCL \ quantity(ml) \times Nm \ (0.05)}{MCL \ quantity(ml) \times Nm \ (0.05)}$	5)×0.014×
sample Weight	~100
5 3 Fat determination ·	

5.3 Fat determination:

The percentage of fat in minced chicken meat samples was estimated according to the AOAC 14 method) using the Soxhlet extraction system, then weighed 1 g of dried ground meat and placed it in a lid of the device (thimble) whose weight is known in advance. Then 200 ml of the solvent (Diethylether) was added to it, and the extraction process lasted about (4-6) hours. The solvent was collected from the apparatus and the thimble was taken out. It was placed in an electric oven for half an hour at 80°C to get rid of the solvent residue from the beaker, then weighed the dried thimble after cooling, and the percentage of fat was calculated according to the following equation:

Fat	was	estimated	according	to	the	following
percentage%=	equa					
Weigh the thimble with the sample before extraction – Weigh	the thi	mble with the	sample after	extr	actior	ı
sample weight	RES	ULTSAND	DISCUSS	ION	:	

×100

4 Ash determination

The percentage of ash in minced chicken meat samples was estimated based on the AOAC method, (13) by taking 2 gm in a ceramic jar with a known weight in advance and then transferring it to the incineration oven at a temperature of $525 \degree C$ for 12 hours or until the weight is stable and a white or gray powder is obtained after the incineration is completed. The weights were fixed, then the weight of the ashes

1- Chemical qualitative detection of some active groups in clove buds extract:

The results of chemical and qualitative detection of some active compounds are shown in Table (3). Clove extract contains many effective chemical compounds, which include phenols, resins, saponins, tins, flavonoids, glycosides and alkaloids, and these compounds have a significant effect in prolonging the shelf life of foods (15).

Effective compound	Alkaloids	Glycosides	flavonoids	tatinas	soaps	resins	phenols
Detection result	+	+	+	+	+	+	+

Presence of active compounds in clove alcohol extract

Previous studies indicated that clove buds contain many effective compounds. When studying the quantitative chemical determination of the compounds of the phenolic extract of the clove plant and evaluating its inhibitory effect on some bacterial isolates contaminated with food, Hashem et al. (16) found that clove extract contains phenols, resins, tins, soaps and flavonoids. glycosides and alkaloids. Jaafar and Khairallah (17) also showed that carnation flowers contain effective compounds such as glycosides, flavonoids and resins.

2- Chemical composition of chilled minced chicken meat:

1-2 moisture moisture

The effect of the interaction between different treatments and the duration of cold storage at a

temperature of 4° C on the moisture content of minced chicken meat has been shown in Table (4).

The results showed a significant increase at (p < p)0.05) in the moisture content of treatment B4 (zinc nanoparticles at a concentration of 20 mg/ml with the highest concentration of clove alcoholic extract 125 mg/ml at a ratio of 1-1) in the first day of cooling, and its percentage was (72.77%). In comparison with the control treatment A (no addition), the moisture content was (64.82). It was also noted from the table that there was a significant increase in the moisture content of treatments B2, B1, B and B3, where their values were at 67.45, 66.01, 68.20 and 68.90%, respectively, compared to its value for the control when stored for 0 days and the results in Table (4) for the treatments when stored for 5 days showed a significant increase (p < 0.05) between treatments B3, B4, B2, B1, B whose values were at 67.97, 65.65, 66.83, 68.19, 69.20%, respectively, in comparison with the relative moisture of the control officer, which was valued at (64.45%) As for the storage period of 10 days, there was also a significant increase (p < 0.05) in the moisture content of treatments B4, B3, B2, B1, B % and it was at 67.69, 64.82, 66.53, 67.76, 68.90 %, respectively, in comparison with the moisture content of the control sample A, which Its value was at (63.86%). As for the effect of the storage period, the results of the statistical analysis indicated a significant decrease between periods, where the percentage of moisture was at its highest level in a period of 0 days, then it began to decrease with the passage of storage

days until it reached its lowest percentage in the period of 10 days. This is consistent with the findings of Al-Alwani (18) in adding rosemary to chilled ground beef, and Siraj study (19), which studied the effect of using natural additives rich in catechins and lycopene on some traits of cold-stored buffalo meat. indicated a decrease in moisture content. The percentage of dry matter was high, and Al-Jubouri's study (20) found that the moisture content of chicken meat kept in refrigeration for 14.7.1 days was 72.94, 72, 04, 70.72 percent, respectively. Al-Rubaie et al. (2007) indicated, The moisture content of broiler chicken meat stored in refrigeration for 0,6 days was 76.60, 66.07 and 65.84%, respectively.

Table (4) Moisture percentage for different treatments when stored in cold storage for periods of(10,5,0) days

	Treatments	Storage period (day)		
Traits	Traiments	0	5	10
	Α	0.01 <u>+</u> e 64.82	0.01 <u>+</u> 64.45 f	0.05 <u>+</u> e 63.86
Moisture	В	0.05 <u>+</u> 68.20 b	<u>0.32</u> <u>+</u> 67.97 с	0.01 <u>+</u> 67.69 b
+	B1	0.04 <u>+</u> 66.01 d	0.03 <u>+</u> 65.65 e	0.01 <u>+</u> 64.82 de
standard	B2	0.01 <u>+</u> 67.45 c	0.02+66.83 d	0.02 <u>+</u> 66.53 c
error	B3	0.26 <u>+</u> 68.90 b	0.01 <u>+</u> 68.19 b	0.04 <u>+</u> 67.76 b
	B4	0.1 <u>+</u> 72.77 a	0.11 <u>+</u> a 69.20	0.26 <u>+</u> 68.90 a

* A control treatment without adding, B (ZN NPs 20 mg/ml), B1 (clove alcoholic extract 100 mg/ml) and B2 (clove alcoholic extract 125 mg/ml) B3 (clove alcoholic extract with ZN NPs lower concentration 1-1) B4 (Alcoholic clove extract with ZN NPs top concentration 1-1).

2-2 Protein:

Table (5) shows the effect of the period of cold storage at a temperature (4°C) on the percentage of protein, as the results showed a significant increase (p < 0.05) in the percentage of protein for treatments B4, B3, B2, B1, B, as it was at 18.15, 17.81, 17.13, 19.48, 19.30%, respectively, compared to the protein percentage of the control sample, which was at 17.02% in the first period of storage and the results showed, after storage for 5 days, that there was a significant increase in the percentage of protein, and the highest value of treatment B4 () was at 19.9% compared to the control sample, which was 17.59% .A significant increase was also observed in the last storage period for 10 days at (p < 0.05) in the percentage of protein, as the highest value of the treatment was (B4 nano zinc at a concentration of 20 mg/ml with the highest concentration of clove alcohol extract 125 mg/ml at a ratio of 1-1) when 20.92% compared to the percentage of protein for control sample A, which was at 18.59, while the percentage of protein in the rest of the treatments B3, B2, B1, and B was valued at 20.07, 18.73, 18.62, 19.58 %, respectively.As for the effect of the storage period, the results of the statistical analysis indicated that there was a significant increase (P < 0.05) between the periods, as the percentage of protein was at its lowest level in the period 0 days and then began to rise with the passage of storage days until it reached its highest percentage in the period of 10 days. Some previous studies indicated a high percentage of protein due to the effect of adding some natural additives to meat and meat products when preserving by refrigeration or top freezing (18). This is consistent with the results of Huang, (21) when it used lotus flower and barley extracts and added them to pork, where this led to a significant increase in the

proportion of protein for the treatments to which it was added compared to the control treatment and it also agreed with Al-Alwani (18), The results also agreed with the findings of Hong et al. (2015), as the percentage of protein in fresh chicken breast reached 26.67% and rose to 28.38% on the fourteenth day of cold storage at a temperature of 4°C. Al-Jubouri's study (20) found that the protein content of chicken meat stored in refrigeration for a period of 14.1.7 was 21.87, 22.97 and 23.76%, respectively, and Al-Rubaie et al. (22) confirmed that the protein content of broiler chicken meat stored in refrigeration for a period of 0,3 6 days were 20.43, 20.7 and 21.02%, respectively. The results of the study also agree with the findings of the study of Siraj (19) and Al-Alwani (18).

Table (5) the percentage of protein for different treatments when stored in cold storage for periods of (10,5,0) days

c	Treatments	Storage period (day)			
⁵ Trait	Treatments	0	5	10	
	Α	<u>+</u> 0.0 17.02 e	<u>+</u> 0.02 17.59 f	<u>+</u> 0.0 18.36 e	
prote	В	<u>+0.02</u> 18.15 b	+0.05 18.90 c	+0.02 19.58 b	
n +	B1	<u>+0.0 17.13 d</u>	<u>+0.0</u> 18.51 e	+0.0 18.62 de	
stand	B2	<u>+</u> 0.01 17.81 c	<u>+0.0 18.73 d</u>	<u>+0.0 18.73 c</u>	
ard	B3	<u>+0.0 19.48 b</u>	+0.0 19.85 b	<u>+0.01 20.07 b</u>	
erro	B4	+ 0.23 19.30 a	19.90 +0.0 a	+0.01 20.92 a	

* A control treatment without adding, B (ZN NPs 20 mg/ml), B1 (clove alcoholic extract 100 mg/ml) and B2 (clove alcoholic extract 125 mg/ml) B3 (clove alcoholic extract with ZN NPs lower concentration 1-1) B4 (Alcoholic clove extract with ZN NPs top concentration 1-1).

2-3 fat

The difference between the treatments and the differences in the percentage of fat were shown in Table (6), where the interaction between the different treatments and the duration of cold storage at a temperature (4°C) was when estimating the percentage of fat in the different treatments and as shown in the table, where the results showed a significant effect in the decrease in the percentage of fat at 0.05) < p) for treatment (B4) nanozinc at a concentration

of 20 mg/ml with the highest concentration of clove alcohol extract 125 mg/ml at a ratio of 1-1) when storing for (0) days, as its percentage was (9.17%) compared to the control sample (no addition of The percentage of fat in it was (9.85%).As for the storage period for (5) days, the results showed a significant decrease (p < 0.05) between treatments B3, B2, B1, B, B4, and their value was at 10.31, 11.13, 10.71, 10.13, 10.01%, respectively when compared with the treatment of Control A, which was at 11.62%.While the results of the statistical analysis of the percentage of fat when stored for 10 days indicated that there were significant differences at (p < 0.05), a significant decrease in the treatments was noted, and the lowest value of the B4 sample was at 10.99% compared to the fat percentage of the control sample A, which was at 13%. The difference in storage durations (10,5,0) had a significant effect on the percentage of fat, as it was noted that the lowest level of the percentage of fat was at storage (0) days and then began to rise with the passage of storage days until it reached its highest level at 10 days in storage.Some previous studies indicated a decrease in the percentage of fat in treatments to which some medicinal plants or their extracts were added. AL-Rubeii, (23) indicated that lambs fed on rosemary extract recorded a significant decrease (<0.01 p) in the percentage of fat compared with those fed on black bean and blackberry control, Al-Alwani (24) indicated that adding rosemary and carnosic acid to chilled ground beef recorded a significant decrease (0.01 < p) in the percentage of fat compared to the control treatment, and the reason for this was due to the

addition of clove extract to contain Eugenol, which is one of the The main compounds of cloves Studies have confirmed that it possesses antioxidant activity, as well as the fact that the clove plant contains many effective compounds, including Vanillic, Kaempferol, as well as containing many effective groups, including tannins, saponins, alkaloids and alpha phenols (18). As well as the fact that these additives are nano-extracts. which increases their effectiveness as an antioxidant compared to the extracts of the same substance and in its natural form ((24). This is considered normal. As the storage period progresses, the moisture decreases and the dry matter increases, which includes both protein, fat and ash. This is consistent with what was found by the hybridization study (25) that the percentage of fat in minced chicken pellets during cold storage was 9.85% for fresh control samples, and the percentage increased to 11. .31% on the fourth day of refrigerated storage at 4°C. The findings of the study of Al-Dhahiri (27) and Al-Alwani (18) are also in agreement.

Table (6) the percentage of fat for different treatments when stored in cold storage for periods of
(10,5,0) days

	Storage period (day)		Treatments		
10	5	0		Traits	
0.0 <u>+</u> 13.0 a	0.0 <u>+</u> 11.62 a	0.57 <u>+</u> a 9.85	Α		
0.0 <u>+</u> 11.35 с	0.01 <u>+</u> 10.31 b	0.0 <u>+</u> 9.38 a	В		
0.0 <u>+</u> 12.52 b	0.05 <u>+</u> 11.13 a	0.04 <u>+</u> 9.65 a	B1	fat +	
0.0 <u>+</u> 11.73 с	0.0 <u>+</u> 10.71 b	0.01 <u>+</u> 9.40 a	B2	standard	
0.31 <u>+</u> 11.28 с	0.0 <u>+</u> 10.13 b	0.01 <u>+</u> 9.19 a	B3	error	
0.0 <u>+</u> 10.99 d	0.0 <u>+</u> 10.01 b	0.0 <u>+</u> 9.17 a	B4		

* A control treatment without adding, B (ZN NPs 20 mg/ml), B1 (clove alcoholic extract 100 mg/ml) and B2 (clove alcoholic extract 125 mg/ml) B3 (clove alcoholic extract with ZN NPs lower concentration 1-1) B4 (Alcoholic clove extract with ZN NPs top concentration 1-1).

2-4 Ash

The results in Table (7)showed that the effect of storage for different periods on the percentage

of ash in the studied samples, as it was noted from the table that there was a significant decrease in the percentage of ash for treatment B4 (nano clove extract, the highest concentration 1-1) when stored for a period of (0) days in refrigeration, and its percentage was at 0.65% compared to the control sample A (without addition), which was 0.91%, It was noted that there was a significant decrease in the percentage of ash for treatments B, B1, B2 and B3, whose value was at 0.78, 0.85, 0.81 and 0.69%, respectively, compared to its value for the control sample. The effect of the storage period for (5) days also had a significant effect, as a decrease in the values of treatments was observed, and the lowest value was for treatment B4, which was at 0.70%, compared with treatment A, which was at 1.11%. As for storage for a period of 10 days, there was also a significant decrease (P < 0.05) in the ash percentage for treatments B, B1, B2, B3 and B4, and its value was at 0.98, 1.09, 1.05, 0.92 and 0.87%, respectively, compared to the ash percentage for a sample. Control A (no addition), which was valued at (1.22%). As for the effect of the storage period, the results of the statistical analysis indicated that there was a

significant effect (P<0.05) between the periods, where the percentage of ash was at its lowest level in the first period and then began to rise with the passage of storage days until it reached its highest percentage in the period of 10 days.Previous studies indicated a lower percentage of ash in treatments added to some plant extracts compared to the control treatment Studies have previously indicated a (18).decrease in the percentage of ash in the treatments to which some medicinal plants or their extracts were added compared to the control treatment, where previous studies indicated a decrease in the percentage of ash in the treatments to which some plant extracts were added compared with the control treatment (18) and (27). This is due to the progression of the storage period, where the Moisture decreases and the dry matter increases, which includes protein, fat and ash, and this agrees with AL-Rubeii and others (23) in their study, as well as Al-Alwani (18).

Table (7) Ash percentage for different treatments when cold storage for (10,5,0) days

S	torage period (day)		Treatments	
10	5	0		Traits
<u>+</u> 0.0 1.22 a	<u>+</u> 0.01 1.11 a	<u>+</u> 0.0 0.91 a	Α	
<u>+</u> 0.0 0.98 ab	<u>+ 0.0 0.83 c</u>	<u>+</u> 0.0 0.78 b	B	A . L
<u>+0.0 1.09 a</u>	<u>+</u> 0.0 1.0 a	<u>+</u> 0.0 0.85 a	B1	Ash +
<u>+</u> 0.0 1.05 a	<u>+</u> 0.01 0.92 b	<u>+0.01</u> 0.81 a	B2	standar d error
<u>+ 0.0 0.92 a</u>	<u>+</u> 0.0 0.81 d	<u>+ 0.0 0.69 c</u>	B3	a error
<u>+ 0.0 0.87 c</u>	<u>+</u> 0.0 0.70 d	<u>+0.01 c 0.65</u>	B4	

A control treatment without adding, B (ZN NPs 20 mg/ml), B1 (clove alcoholic extract 100 mg/ml) and B2 (clove alcoholic extract 125 mg/ml) B3 (clove alcoholic extract with ZN NPs lower concentration 1-1) B4 (Alcoholic clove extract with ZN NPs top concentration 1-1).

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