THE POLYMORPHISM OF ZP1 GENE IN AFRICAN OSTRICH

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ABSTRACT

This study was conducted to characterize the nucleotide variations within the coding region of zona pellucida sperm binding 1 (ZP1) gene in African ostrich's populations that are being raised in Iraq. After extracting genomic DNA, designing specific PCR primers, PCR, SSCP, and sequencing experiments were performed. The SSCP patterns were identified and compared. Two genotypes were observed with two alleles within ZP1 amplified locus. This study may determine the pattern of polymorphism within ZP1 gene to estimate its important effect on African ostrich breed.

Keywords: genotyping, ostrich, *in vitro*, SSCP

INTRODUCTION

ZP1 is one of the genes belonging to the ZP gene family which is made up in birds of ZP1, ZP2, ZP3 ZP4, ZPD, ZPAX [1,2]. ZP1 gene is found in chickens on chromosome five [3]. The zona pellucida (ZP) matrix that encircles vertebrate oocytes is also participated in the binding of sperm and provides assistance to the oocyte as it moves down the oviduct. The parallel structure of zona pellucida in the avian species is the perivitelline membrane. Its commonly known that the ZP is cosists of three major glycoproteins encoded by ZP1, ZP2, and ZP3 that are highly expressed in the oocytes. Nevertheless, chicken ZP1 is synthesized in the liver under the control of oestrogene and transported through blood circulatory system [4]. The Zona Pellucida gene family is involved in egg envelope formation throughout the vertebrate lineage. These genes evolve rapidly, with differences occurring in the type and number of copies, causing lineage and species-specific gamete interactions that may originate reproductive barriers and lead to speciation. In contrary to monospermic mammals, birds and reptiles undergo physiological polyspermy, without detrimental consequence on later development [5]. The function of ZP1 is similar in all species and it is mainly structural [3,6] forming disulfide bonds with ZP2/ZP3 filaments [7,8]. Dimeric ZP1 might be responsible for stimulate sperm activation [9]. ZP1 is responsible for acrosome reaction [10, 11]. Hence, ZP1 genetic locus may be powerful marker for assessment of genetic variation. To resolve its possible polymorphism, several post-PCR techniques were usually employed to detect its polymorphism, as in single stranded conformation polymorphism, or SSCP [12]. Its ability possibly attributed to its ability of the detection of unknown mutations [13]. Studies in ostriches regarding to ZP1 gene are rare, since the polymorphisms of ZP1 gene have been highlighted in very few cases. Very little genetic data is currently available about this genetic fragment regarding its genetic polymorphism. Therefore, the main aim of this study is to analyze the variation of the ZP1 gene by utilizing the *in vitro* genotyping PCR-SSCP-sequencing means.

MATERIALS AND METHODS

Ostrich's resources

The study was performed on 69 African ostriches in several portions of Iraq. The studied ostrich population was raised in several regions in the middle and southern parts of Iraq. DNA samples were collected from the feathers using a universal protocol [14]. The

extracted DNA was assessed by 0.8% agarose gel electrophoresis and quantified by a nanodrop.

PCR Design

One pairs of PCR specific primers was designed using the NCBI primer BLAST server. One PCR amplicon was selected, in which the sequence of forward primer was 5′- GCTTTAGCTCTTCCTGGGCA-3′, while the sequence of reverse primer was 5′- CCAGCTTGAGAGTGGGTCTG-3′.

PCR Analysis

The PCR reaction was conducted using a Bioneer PCR premix. The total PCR mix consists of 20µl of PCR premix constituents (1 unit of *Top* DNA polymerase, 30 mM of KCl 250, 1.5 mM of $MgCl_2$, µM of dNTPs, 10 mM of Tris-HCl of pH 9.0) (Bioneer – South Korea). The optimum annealing temperatures were determined empirically in our extracted genomic DNA template using gradient PCR thermocycler (Eppendorf). The PCR program was: initial denaturation at 94°C for 5 min, followed by 30 cycles of denaturation at 94°C for 30 sec, annealing at 63.0°C for 30 sec, and elongation at 72°C for 30 sec, and was concluded with a final extension at 72°C for 5 min. PCR products were assessed by agarose gel electrophoresis. All SSCP non-suitable amplicon bands were eliminated from downstream experiments.

SSCP Analysis

The post-PCR SSCP genotyping experiments were conducted according to Al-Shuhaib' protocol with some modifications [14]. Multiple parameters have been modified to get the best reliable results. Briefly, 2.5 µl of each amplification product was mixed with equal volume of SSCP denaturing loading buffer. The PCR amplicons were then heat-treated at 95°C and incubated on ice for about 10 min each. Then, denatured samples were separated in vertical mini-gel format, with 1 mm of gel thickness (Junyi-Dongfang – China). Denatured amplicons were electrophoresed of 8% acrylamide/bis (37.5:1) (with 7% glycerol, and 1x TBE buffer) at constant conditions (250 Volt, 125 mA, for 330 min) at laboratory conditions. SSCP gels were fixed and stained by silver staining technique [15].

DNA Sequencing

Each different SSCP samples' pattern for the amplified *ZP1* gene fragments was purified and both termini were commercially sequenced (Macrogen, South Korea). The ostrich reference sequences of *ZP1* locus, was retrieved from NCBI websites (NW_009272030.1). Then, the sequenced genotypes were analyzed and compared with the NCBI referring sequences using *BioEdit* tool (DNASTAR, Madison, USA).

RESULTS AND DISCUSSION

In the current study, the single nucleotide polymorphisms (SNPs) of the *ZP1* gene were detected by PCR-SSCP and DNA sequencing methods. The observed genetic variations of SSCP gels indicate the detection of two different genotypes AB and BB, with two alleles A and B (Fig. 1). Sequencing results certified these two different SSCP genotypes since only one SNP (n.131376C>T) were detected in the AB genotype while two SNPs were observed in the BB genotypes (n.131376C>T and n.131662G>A) (Fig. 2). Thus, both genotypes share one SNP (n.131662G>A) (Table 1). Thus, it was demonstrated through the *in vitro* study portion of this fragment that *ZP1* genotyping through PCR-SSCP-sequencing can explore this kind of variability. Interestingly, the highlighted fragment of *ZP1* locus of the AB genotype has a higher polymorphic status and may be considered as a mutational site, or the highest polymorphic site, leading to a remarkable diversity in such organisms. The observed high genetic differences in the *ZP1* locus of might be due to several factors including the location, type, or even the climate of the breed used as the nature of polymorphisms is potentially highly reliable on these factors in the resolution of its final genetic variation status. However, the variations in the breeding styles among different regions around the world have led to the suggestion that genetic differentiation between countries may exist as a potential consequence of the genetic drift, local selection, environmental effect, and genetic isolation that couldn't be eliminated from this interpretation.

Table 1. the ZP1 gene nucleotide replacements and types among SSCP haplotypes and their reference NCBI sequence of the studied African ostrich population. The letter "n" refers to the sample sizes of genotyped animals.

PCR fragment /Gene	Nucleotide position in the PCR fragment	Nucleotide change in the NCBI reference sequence	Genotype and sample size	Amino acid change	Type of SNP
ZP-1	254 540	n.131376C>T n.131662G>A	AB(n 6) BB(n 63) and AB(n 6)	- p.S25F	Synonymous Missense

Fig. 1. PCR-SSCP electrophoresis pattern of *zp1* PCR fragment in the African ostriches. Two genotypes were observed in this locus AB and BB, with two alleles A and B.

Our analysis data showed that African ostrich breed can be differentiated by SSCP using the previously described *ZP1* genetic fragment. In conclusion, the results of our study suggest that the current *in vitro* genotyping technologies provide an indicator for *ZP1* gene to be as markers of choice in the immigrated African ostrich breed. This marker may be utilized at the nearest future investments of the genetic polymorphism analysis of such organisms in an efficient and cost-effective manner.

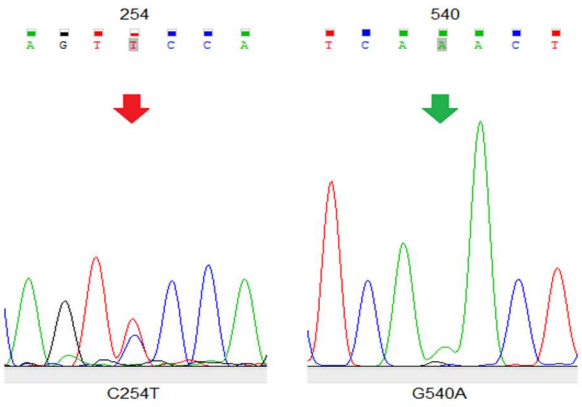

Fig. 2. chromatogram pattern of the two observed SNPs in *zp1* genetic locus.

REFERENCES

[1] Goudet G, Mugnier S, Callebaut I, Monget P. (2008). Phylogenetic analysis and identification of pseudogenes reveal a progressive loss of zona pellucida genes during evolution of vertebrates. Biol. Reprod. 78:796–80.
[2] Hughes DC (2007). ZP genes in avian species illustrate the dynamic evolution of the vertebrate egg envelope. Cytogenet. Genome Res. 117:86–91.
[3] Jacqueline smith , Lin R. Paton, David C. Hughes, and David W. Burt (2005). Isolation and Mapping the Chicken Zona Pellucida Genes: An Insight Into the Evolution of Orthologous Genes in Different Species. molecular reproduction and development 70:133–145.
[4] Bausek N, Waclawek M, Schneider WJ, Wohlrab F (2000). The majorchicken egg envelope protein ZP1 is different from ZPB and is synthesized in the liver. J Biol Chem 275:28866–28872.
[5] Imran Khan (2014). Using genomic and proteomic information to characterize the evolution of genes involved in development and adaptation in vertebrates under differential conditions of selective pressure. PhD Thesis.F.C.U.PORTO.
[6] Gupta SK, Bhandari B, Shrestha A, Biswal BK, Palaniappan C, Malhotra SS, Gupta N. (2012). Mammalian zona pellucida glycoproteins: structure and function during fertilization. Cell Tissue Res. 349:665–678.
[7] Jovine L, Darie CC, Litscher ES, Wassarman PM(2005). Zona pellucida domain proteins. Annu. Rev. Biochem. 74:83–114.
[8] Kubo H, Shiga K, Harada Y, Iwao Y (2010). Analysis of a sperm surface molecule that binds to a vitelline envelope component of Xenopus laevis eggs. Mol. Reprod. Dev. 77:728–735.
[9] Okumura H, Kohno Y,Iwata Y, Mori H, Aoki N, Sato C, Kitajima K,Nadano D, Matsuda T. (2004). A newly identified zona pellucida glycoprotein, ZPD, and dimeric ZP1 of chicken egg envelope are involved in sperm activation on sperm-egg interaction. Biochem. J. 384:191–199.
[10] Meslin C, Mugnier S, Callebaut I, Laurin M,Pascal G, Poupon A, Goudet G, Monget P (2012). Evolution of Genes Involved in Gamete Interaction: Evidence for Positive Selection, Duplications and Losses in Vertebrates. PLoS ONE 7:e44548.
[11] Mao H-T,Yang W-X (2013).Modes of acrosin functioning during fertilization. Gene 526:75–79.
[12] Orita M, Iwahana H, Kanazawa H, Hayashi K, Sekiya T (1989): Detection of polymorphisms of human DNA by gel electrophoresis as single-strand conformation polymorphisms. Proceeding National Academy of Sciences of USA, 86, 2766-2770.
[13] Gasser RB, Hu M, Chilton NB, Campbell BE, Jex AJ, Otranto D, Cafarchia C, Beveridge I, Zhu X (2006): Single-strand conformation polymorphism (SSCP) for the analysis of genetic variation. Nature Protocols, 1(6), 3121-3128.
[14] Al-Shuhaib, MBSA (2017). A universal, rapid, and inexpensive method for genomic DNA isolation from the whole blood of mammals and birds. J. Genet. 96, 171-176.
[15] Byun SO, Fang Q, Zhou H, Hickford JGH (2009). An effective method for silver-staining DNA in large numbers of polyacrylamide gels. Anal Biochem 385, 174-175.

GENDER AND ITS EFFECT ON SOME PHENOTYPIC TRAITS IN IRAQI AWASSI SHEEP

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ABSTRACT

This study was conducted on Awassi Sheep to investigate the effect of gender on some phenotypic traits. Phenotype measurements were taken from 209 sheep slaughtered at Abattoirs of Babylon and Karbala in provinces of Babylon and Karbala. Body Weight (B.W.), Back Fat Thickness (B.F.T.), Body Length (B.L.), Fat Tail Weight (F.T.W.) and Abdominal Fat (A.F.) were recorded at slaughter. A total of 209 individuals from Awassi sheep (160 male and 49 female)were examined in this study. From the jugular vein approximately 5 ml blood was collected then centrifugation at 3000 rpm for 15 min to separate sera were used for measurement of lipid profile while the remaining were stored frozen at -20 ºC until hormonal assayed. The results refer to the presence of the significant differences ($P< 0.05$) in body length, abdominal fat and dressing in male than female group (80.81 ± 1.48), (2.146 ± 0.06) and (54.38 ± 0.61) respectively. While female group showed significant differences ($P< 0.05$) in the fat tail than male (3.495 ± 0.82). There were significant differences ($P< 0.05$) in TSH concentration in male group than female group (0.644 ± 0.19) , (0.523 ± 0.10) respectively. The results of the present study showed that there were no significant differences ($p > 0.05$) in lipid profile levels between male and female groups. The study suggests males have heavier body length, dressing and TSH hormone than females in Awassi sheep.

Keywords: Gender, Phenotype measurements, production traits, Iraqi sheep.

INTRODUCTION

The native breeds of sheep in Iraq include the Awassi 58.2 %, Arabi 21.8% and Karadi sheep 20%. Awassi is one of the main sheep breeds in Iraqi regions. These are all fat-tailed, carpet-wool production with some potential to produce milk. Although these breeds are discriminated by low of each growth, fertility, and milk production, their ability to survive and reproduce under the condition of drought and extreme climate fluctuations is notable [1, 2]. Genetic, nutritional and environmental factors affect growth which is considered a complex mechanism of metabolic and physiological [3]. External factors such as nutrition are necessary to supply metabolizable energy for animal growth and body fat deposition. There are also many intrinsic factors implicated in growth regulation and deposition of body fat, including hormonal as well as several genetic factors. Growth hormone axis genes influence on a variety of physiological parameters for instance appetite control, growth, body composition, aging, and fertility as well as immune responsiveness [4].Males have heavier body weights than females due to the natural hormonal variation in most animal species [5]. The most important adaptation in the animal was the ability to use body fat during the times of stress and selection in favor of fat deposition that associated with overall productivity of the ewes may be considered a breeding objective aimed at reducing animal losses during 'unfavorable' periods. In young rams found that large fat-tail may interfere with the mating and may decrease the fertility [6]. So, this study aims to investigate the effect of gender on some phenotypic traits in Awassi sheep.

MATERIALS AND METHODS

Sheep populations and Sampling

This study was conducted at the College of Agriculture / AL-Qasim Green University/department of Animal Resources for the period October /2016 to June / 2017 on Awassi Sheep. Phenotype measurements were taken from 209 sheep (160 male and 49 female) slaughtered at Abattoirs of Babylon and Karbala in provinces of Babylon and Karbala. Body Weight (B.W.), Back Fat Thickness (B.F.T.), Body Length (B.L.), Fat Tail Weight (F.T.W.) and Abdominal Fat (A.F.) were recorded at slaughter by the methods described by [7] and [8].

Blood Serum and Hormonal Analysis

From the jugular vein approximately 5 ml blood was collected then centrifugation at 3000 rpm for 15 min to separate sera were used for measurement of lipid profile while the remaining were stored frozen at -20 ºC until hormonal assayed. The quantitative sandwich enzyme immunoassay technique was used to determine the serum TSH, T3, and T4. ELISA assay was conducted in the College of Sciences / University of Babylon. An enzymatic method was used to determine serum lipid profile.

Statistical Analyses

The Statistical Analysis System [19] was used to effect of gender in study parameters with applied of General Linear Model-GLM procedure and [10] multiple range test (ANOVA) was used to significant compare between means. Estimate of the correlation coefficient between some variables in this study.

RESULTS AND DISCUSSION

The results of this study refer to the presence of the significant differences (P< 0.05) in body length, abdominal fat and dressing in male than female group (80.81 ± 1.48), (2.146 ± 0.06) and (54.38 ± 0.61) respectively. While female group showed significant differences (P< 0.05) in the fat tail than male (3.495 ± 0.82), as shown in Table (1).

Table (1). Effect of gender in body parameters in Iraqi sheep

Gender	No.	Mean ± SE					
		Body length (cm)	Fat tail (kg)	Abdominal fat (kg)	Back fat (kg)	Net weight (kg)	Dressing (%)
Male	135	80.81 ± 1.48 a	2.020 ± 0.07 b	2.146 ± 0.06 a	2.972 ± 0.52 a	26.51 ± 0.33 a	54.38 ± 0.61 a
Female	52	77.57 ± 1.66 b	3.495 ± 0.82 a	1.874 ± 0.11 b	2.134 ± 0.09 a	25.67 ± 0.47 a	49.98 ± 0.79 b
Level of sig.	---	*	*	*	N.S.	N.S.	*

* (P<0.05), N.S.: Non-Significant.

-Means having with the different letters in same column differed significantly.

Information about the morphological structure and development ability of the animals can be obtained from the body measurements that are reflecting the breed standards and represent the important data sources. Many factors such as breed, gender, yield type, and age can be affected by body measurements.

The most common parameters used for body measurements in sheep are; body length, body weight and live body weight that can exert a significant effect in several of economically important traits in farm animals [11].

The natural hormonal variation in most animal species made males have heavier body weights than females [5]. In young rams found that large fat-tail may interfere with the mating and may decrease the fertility so the most important adaptation in animal was able to use body fat during the times of stress and selection in favour of fat deposition that associated with overall productivity of the ewes may be considered a breeding objective aimed at reducing animal losses during 'unfavorable' periods [6].

The gender had notable influences on body composition traits. The male was heavier than the female of all ages [12]. Values of average live weight at slaughter, hot carcass weight, and muscle area were found higher in males than females. While no effects for gender on dorsal fat depth were observed in the suckling lamb breeds [13]. No adverse effect on the cold environment on either growth performance or carcass quality for lambs that were usually slaughtered at live weights of 45 kg for females and 50kg for males[14]. There were significant differences ($P< 0.05$) in TSH concentration in male group than female group (0.644 ± 0.19), (0.523 ± 0.10) respectively. While no significant differences ($P> 0.05$) in T3 and T4 concentration between groups were observed (Table 2).

Table (2). Effect of gender in level of hormones in Iraqi sheep

Gender	No.	Mean ± SE		
		T4 (IU/ml)	T3 (IU/ml)	TSH (IU/ml)
Male	46	7.15 ± 0.82 a	2.926 ± 0.18 a	0.644 ± 0.19 a
Female	39	6.40 ± 0.86 a	3.311 ± 0.26 a	0.523 ± 0.10 b
Level of sig.	---	N.S.	N.S.	*

* ($P<0.05$), N.S.: Non-Significant.
-Means having with the different letters in same column differed significantly.

Sexual hormones in sheep males promote the greater activity of the thyroid gland than female made them had higher concentrations of thyroxine in comparison to females similar with humans, which exhibited higher thyroxine levels in men than women, so the gender is the main factor that interferes with the secretion of thyroxine in both sheep and human [15].

Both nutrition and thyroid hormone levels in blood found to be affected by endogenous factors including age, gender, physiological conditions and environmental factors. Thyroid gland synthesizes thyroid metabolic hormones (TH) including Triiodothyronine (T3) and Thyroxin (T4) that influences on most cells of the body's organ by regulating growth and lipid metabolism and improves tissue oxidation of fatty acids[16]. Normal physiological status of the animal can be evaluated from the hematological and biochemical values that are efficient for evaluation of health condition [17]. Several factors including gender, breed, age, stress, diet, physiological status and the laboratory methodology are influenced by the levels of blood parameters [18]. While in Turkoman horses, sheep and water buffaloes, no significant effect was found for gender on the serum thyroid hormones, triglyceride, and cholesterol [19]. As well as [20] and [21] studied the correlations between thyroid metabolic hormones and lipid profile level in clinically healthy Iranian male goats and nonpregnant uniparous Iranian ewes, and they found there was no significant relationship between thyroid hormones and serum lipid profile. Table (3) refers to the effect of gender in lipid profile in Iraqi sheep. The results of the current study revealed that there were no significant differences ($p > 0.05$) in lipid profile levels between male and female groups.

Table (3). Effect of gender in lipid profile in Iraqi sheep

Gender	No.	Mean ± SE				
		Cholesterol (mg/dl)	Triglyceride (mg/dl)	HDL (mg/dl)	VLDL (mg/dl)	LDL (mg/dl)
Male	46	92.87 ± 4.86	107.69 ± 6.56	29.32 ± 2.08	21.57 ± 1.32	45.14 ± 4.27
Female	39	86.89 ± 4.46	104.91 ± 11.17	30.63 ± 2.61	22.08 ± 2.01	43.95 ± 3.82
Level of sig.	---	N.S.	N.S.	N.S.	N.S.	N.S.

N.S.: Non-Significant.

Consistent with this result the values of cholesterol and triglycerides of the Morada Nova sheep were significantly (P> 0.05) not affected by the gender in the study by [22]. As well as [23] showed that gender is not as important determinant to release the total FFA as total fat. Other study revealed that cholesterol concentration and HDL level in males were higher than females but this difference was not significant [24]. Sheep females tend to have higher values for triglycerides than males. VLDL level in females was comparable to those in males with no gender effect on this parameter [24]. In clinically healthy camels, thyroid hormones and triglyceride showed no significant differences between the gender and age groups [25]. Conversely, gender also appears to affect the efficiency of energy use for growth, the net efficiency of the metabolizable energy (ME) utilized for weight gain is (65.5%) for ewes, and (57.6%) for rams this mean that the gains of the ewes contained more fat [26]. In conclusion, we have confirmed in the present study that males have heavier values in body length and dressing also in TSH hormone concentration than females.

REFERENCES

[1] Yousif, M. S.; Al-Barzinji and Othman,G.U. (2013). Genetic Polymorphism in FecB Gene in Iraqi Sheep Breeds Using RFLP-PCR Technique. IOSR Journal of Agriculture and Veterinary Science , 2(4): 46-48.

[2] Al-Thabhawee,A.H.; Samaka,H.M. and Kadhim,H.M. (2014). Detection of (FecB) Gene Polymorphism in Local Sheep Breed at Different Area of Iraq. Mirror of Research in Veterinary Sciences and animals,3 (1): 15-20.

[3] Bhattacharya, T. K. ; Chatterjee, R. N. ; Dushyanth, K.; Paswan, C. and Guru Vishnu, P. (2016). Activin receptor 2A and activin receptor 2B genes in chicken: effect on carcass traits. Journal of Applied Animal Research, 44(1): 480-486.

[4] Moradian,C.; Mohamadi,N.; Razavi-Sheshdeh,S.A.; Hajihosseinlo,A. and Ashrafi, F.(2013). Effects of genetic polymorphismat the growth hormone gene on growth traits in Makooei sheep. European Journal of Experimental Biology, , 3(3):101-105.

[5] Sarti,F.M.; Castelli,L.;Bogani,D. and Panella,F. (2003). The measurement of chest girth as an alternative to weight determination in the performance recording of meat sheep, Italian Journal of Animal Science, 2:123-129.

[6] Esmailizadeh, A. K. ; Miraei-Ashtiani, S. R. ; Mokhtari, M. S. and Asadi Fozi, M.(2011). Growth Performance of Crossbred Lambs and Productivity of Kurdi Ewes as Affected by the Sire Breed under Extensive Production System. J. Agr. Sci. Tech. , 13: 701-708.

[7] Gilbert, R.P.; Bailey, D.R. and Shannon, N.H.(1993).Linear body measurements of cattle before and after 20 years of selection for postweaning gain when fed two different diets.J. Anim. Sci.,71(7):1712-20.

[8] Bahrami, A.; Miraei-Ashtiani, S.R. and Mehrabani-Yeganeh, H.(2012). Associations of growth hormone secretagogue receptor (GHSR) genes polymorphisms and protein structure changes with carcass traits in sheep. Gene,505 : 379–383.

[9] SAS.2012. Statistcal Analysis Syatem, User's Guide. Statistical. Version 9.1 th ed. SAS. Inc. Cary. N.C. USA.

[10] Duncan, D.B. 1955. Multiple Rang and Multiple F-test. Biometrics. 11 :4-42 .

[11] Pesmen,G. and Yardimci,M.(2008). Estimating the live weight using some body measurements in Saanen goats. Archiva Zootechnica, 11:4:30-40.

[12] Baneh,H. ; Hafezian,S.H.; Rashidi,A.; Gholizadeh,M. and Rahimi,G. (2010). Estimation of Genetic Parameters of Body Weight Traits in Ghezel Sheep. Asian-Aust. J. Anim. Sci., 23(2) : 149 – 153.
[13] Perez, P.; Maino, M.; Morales, M.S. ; Kobrich, C. ; Bardon, C. and Pokniak, J.(2007). Gender and slaughter weight effects on carcass quality traits of suckling lambs from four different genotypes. Small Rumin. Res., 70(2-3):124-130.
[14] Pouliot, E´ .; Garie´py, C.; The´ riault, M.; Avezard, C.; Fortin, J. and Castonguay, F. W. (2009). Growth performance, carcass traits and meat quality of heavy lambs reared in a warm or cold environment during .winter. Can. J. Anim. Sci., 89: 229 239.
[15] Ahmed, Z.; Khan, M. A. ; Haq, A. U. ; Attaullah, S. and Rehman, J. U. (2009). Effect of race, gender and age on thyroid and thyroid stimulating hormone levels in North-West Frontier Province, Pakistan. J. Ayub Med. Coll. Abbottabad,21(3): 21 -23.
[16] Medrano, Rodolfo, F. and He J. H.(2016). Advances in Thyroid Hormones Function Relate to Animal Nutrition. Annals Thyroid Res. , 2(1): 45-52.
[17] Opara, M. N.; Udevi, N. and Okoli, I. C. (2010).Haematological Parameters And Blood Chemistry Of Apparently Healthy West African Dwarf (Wad) Goats In Owerri, South Eastern Nigeria. New York Science Journal, 3:68-72.
[18] Kaneko, J.J., Harvey, J.W. and Bruss, M.L. (2008). Clinical Biochemistry of Domestic Animals.6th ed. Academic Press; San Diego (USA). 916p.
[19] Tajik, J.; Nazifi, S.; Badiei, K.; Gholaminejad,M.R. and Naghib, S.M. (2012) . Correlations of serum leptin with lipids, lipoproteins, and thyroid hormones in Water Buffalo (Bubalus bubalis). Comp Clin Path , 21(5): 1013-1017.
[20] Nazifi, S.; Gheisari, H. R. and Shaker, F.(2002). Serum lipids and lipoproteins and their correlations with thyroid hormones in clinically healthy goats. Veterinarski Arhiv, 72: 249−257.
[21] Nazifi, S.; Saeb, M.; Rowghani, E.; Hasankhani, M.; Hasanshahi , F. and Ghafari, N.(2007). Studies on the Physiological Relationship Between Thyroid Hormones, Serum Lipid Profile and Erythrocyte Antioxidant Enzymes in Clinically Healthy Iranian Fat-Tailed Sheep. Bulgarian Journal of Veterinary Medicine, 10 (3): 161 −167.
[22] Carlos ,M.M.L. ; Leite , J.H.G.M. ;Chaves , D.F. ;Vale , A.M. ;Façanha , D.A.E. ; Melo, M.M. and Soto-Blanco, B.(2015). Blood Parameters in the Morada Nova Sheep: Influence of Age, Sex and Body Condition Score. The Journal of Animal & Plant Sciences, 25(4): 950-955.
[23] Saponaro,C.; Gaggini,M.; Carli,F. and Gastaldelli,A.(2015). The Subtle Balance between Lipolysis and Lipogenesis: A Critical Point in Metabolic Homeostasis. Nutrients, 7: 9453–9474.
[24] Sitmo, M.S.(2014). Effect of gender on some plasma biochemical parameters of sheep from Southern Al Jabal Al Akhdar in Libya. Journal of American Science ,10(8):74-77.
[25] Tajik,J.; Sazmand,A.; Moghaddam,S.H. and Rasooli,A.(2013). Serum concentrations of thyroid hormones, cholesterol and triglyceride, and their correlations together in clinically healthy camels (Camelus dromedarius): Effects of season, sex and age. Veterinary Research Forum. , 4 (4) :239 – 243.
[26] Bull, L. S.; Reid, J. T. and Johnson, D. E. (1970). Energetics of sheep concerned with the utilization of acetic acid. J. Nutr. 100: 262-76.

CHEMICAL FABRICATION AND ANTIBACTERIAL EFFECT OF AQUEOUS COLLOIDAL SOLUTION OF SILVER NANOPARTICLE

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ABSTRACT

The submicron particles have shown many innovated properties, which enabled researchers in various fields of science to develop newly manufactured products. Ag NPs among these sub-micron particles have shown new physicochemical properties, besides the well-known antibacterial activity of silver ion, which possesses these particles well-built biocide property. In the present study, Ag NPs have been synthesized by the chemical reduction method and characterized with UV-Vis which shows peak absorbance at 410 nm which confirm the changed color of silver nitrate to yellowish color of silver nanoparticles and by SEM which revealed 50 nm approximately in a spherical shape. *E-coli* as gram -ve and *Staphylococcus aureus* as gram +ve pathogenic bacteria have been used to estimate the cytotoxic efficiency of Ag NPs in different concentrations 60, 70 and 80 mg/ml using hole diffusion method. The antimicrobial activity of Ag NPs was direct proportion with the concentration of Ag NPs. The highest inhibitory zones were obtained in *E-coli* at 80% mg/ml concentration followed by *Staphylococcus aureus* at 80% and 70 % mg/ml concentration. Whereas minimum zone of inhibition has been obtained in *E-coli* was *at* 60% mg/ml concentrations and minimum zone of inhibition in *Staphylococcus aureus* was observed at 60 % mg/ml concentration. The inhibitory effect of silver nanoparticles can be attributed to the high affinity of silver ions which interacts with the sulfur groups of the cysteine which is one of the main components of the cell membrane of the bacteria which leads to the denaturation of the bacteria.

Key words: Silver nanoparticles, borohydride method and antibacterial.

INTRODUCTION

The new physicochemical characteristics of nanomaterials have renowned them compared to their bulk state that has brought the scientists interest in various fields of science. Size depends property is the key issue that nanoparticles relaying on, which resulted in heightened reactivity due to the increased surface area compared with the volume ratio (1). A lot of biocides have been developed such as copper (2), zinc (3), iron (4) and titanium (5). Silver ion has shown distinguished antimicrobial properties in comparison with the above-mentioned antibiotics (6, 7, 8). The antimicrobial property of Ag nps toward many infectious organisms encouraged the researchers to design many industrial products that possess biocidal property such as clothing, food packing, sunscreens, and agriculture and disease management. (9, 10, 11, 12). Many developed methods have been pursued to synthesis Ag nps resulted in a more concentrated and stable nps which in turn outcome in high impact on the growth of bacteria. This notion has attracted many researchers recently to use silver nanoparticles as an effective tool for killing many types of bacteria (13). The nps has the ability to interact with some active site of the cell membrane of the bacteria by various ways which disturb cell viability (14). Generally, the toxicity of nanoparticles is triggered by the induction of non-reduction strain via formation of free radical. However, the mechanisms of its toxic effect

depend mainly on several parameters including composition (15), surface modification (16) and the bacterial species (17). The strong attraction between phosphorous groups and silver nanoparticles interaction key factor of the antimicrobial effect of silver nanoparticles because the cell membrane contains large amounts of proteins that containing sulfur (18). the release of a silver ion from silver nanoparticles can interact with enzyme substrate and causes enzyme activation and react with the phosphorous group of DNA consequential inert of DNA replication all this cause bacterial with abnormal properties so it leads to high bacteria growth or death (19, 17, 20). The high concentrations of silver nps in less than 10 nm in diameter show cell death because of the interaction with cytoplasm components, nucleic acid and cell membrane as well which cause cell lysis (21). The objectives of this study were to synthesize silver nanoparticles, to study their properties as well as to estimate their effects on gram +ve and Gram-ve bacteria. Increasing the surface area of Ag nps compared with the volume ration has increased the ability of interactions between silver ions with diphosphate bond.

MATERIALS AND METHODS

This work has been pursued in the laboratory of microbiology department of genetic engineering, college of the Biotechnology, University of Al-Qasim Green; this work was, the characterization of Ag NPs has been conducted in the Nanotechnology Central Research University of Technology. Silver nitrate Reagent World, sodium borohydride CHD, the reaction media was distilled water. The bacterial chosen medium was nutrient agar media, Uv- Vis, and SEM have been used to characterize Ag NPs.

Synthesis of Ag NPs

Silver nanoparticles were prepared by chemical reductions method or borohydride method (22). 100 ml aqueous solution of $NaBH_4$ 0.001 mM was added drop wisely to 100 ml aqueous solution of 0.015mM $AgNo_3$ and the mixture was vigorously stirred for 30 minutes at room temperature till the color changed to pale yellow. The suspension of Ag NPs was kept in darks flask to protect it from sunlight. Three different diluted forms of Ag NPs have been made; 1 ml of each diluted form has been placed in a foil boat and let dry 72 hours at room temperature in ambient conditions.

Antimicrobial activity of Ag NPs.

The antimicrobial activity of silver nanoparticles has analyzed on pathogenic bacteria *Staphylococcus aurous* Gram-positive and *E. coli* Gram-negative. Hole diffusion method (30). Newly prepared nutrient agar media have been poured into sterile Petri dish plates, permitted to 30 min for solidification. The plates were swabbed with 100 µl of microbial cultures and placed the earlier prepared hole; the test is carried out in triplicates. The plates were incubated at 37°Cl for 24 h, and then the zone of inhibition is measured with the help of a scale land tabulated the results. However, 80, 70 and 60 µg/ml concentration of Ag NPs have been used to measure the antimicrobial activity of Ag NPs where 80 µg/ml have shown significant inhibition zone greater than 70 and 60 µg/ml concentrations in both pathogenic bacteria.

RESULTS AND DISCUSSION

Silver nanoparticles have been synthesized by chemical reduction method (22), yellowish color has been obtained after adding NaBH4 to the aqueous solution of silver nitrate, which indicates the formation of silver nanoparticles; Reduction of silver ion into metal silver nanoparticles was clearly observed by means of a color change of silver nitrate which is colorless to pale yellow after adding sodium borohydride the same color have been obtained by Mavani and Shah 2013. However, the synthesized silver nanoparticles solution was analyzed by UV-Vis spectrophotometer shows absorption peak at 410 nm which confirms the total converting of silver nitrate to silver nanoparticles Figure 1 and table 1.

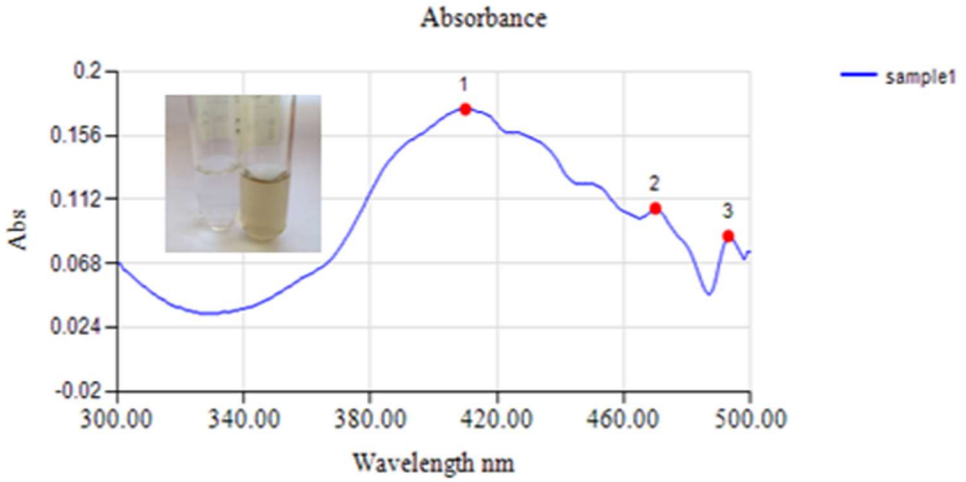


Figure 1. UV–Vis spectra. Formation of yellowish color indicated the synthesis of Ag NPs after adding $NaBH_4$ to $AgNo_2$ along with the intense observed peak at 410 nm for synthesized Ag NPs.

Index	Lembda	Abs
1	410	0.174
2	470	0.106
3	493	0.087

Table 1: Characterization of Ag NPs, Obtained peak at 410 nm

Scanning Electron Microscope has revealed the size and morphology of Ag NPs which shows size rang distributed below 50 nm in diameter and spherical shape Figure 2.

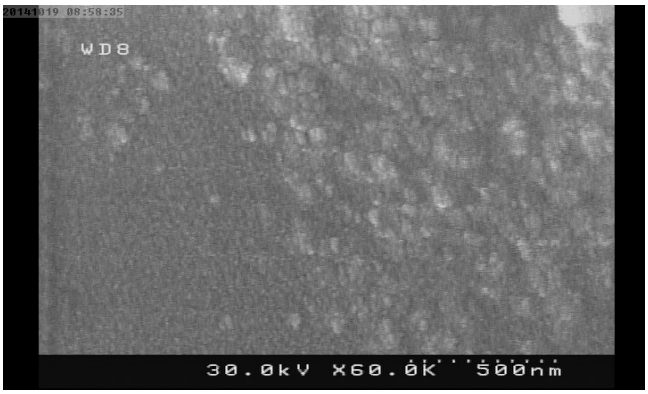
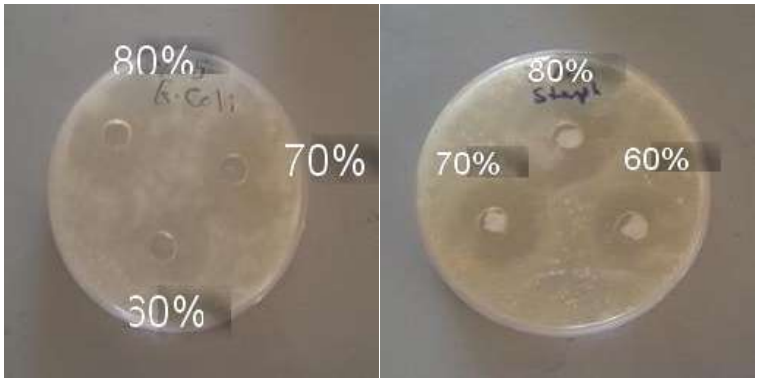


Figure 4.4 Scanning Electron Microscopy (SEM) image of Ag NPs.

The antimicrobial efficiency of Ag NPs have estimated on gram-positive *Staphylococcus aureus* and gram-negative bacterial *E-coli* using hole diffusion method (30). The results were summarized in table 2. The antimicrobial activity of Ag NPs was direct proportion with the concentration of Ag NPs. However, the highest inhibitory zone was observed in *E-coli* at 80% mg/ml concentration followed by *Staphylococcus aureus at* 80% and 70 % mg/ml concentration. While a minimum zone of

inhibition in *E-coli* was observed at 24 mg/ml concentration and minimum zone of inhibition in *Staphylococcus aureus* was observed at 60 % mg/ml concentration figure 3.



A **B**

Figure 3: Zone of inhibition at different concentrations of Ag NPs against A *E. coli* and B *S. aureus*.

Bacteria	Con mg/ml	Zone of inhibition mm	Con mg/ml	Zone of inhibition mm	Conl mg/ml	Zone of inhibition mm
E-coli	60%	24	70%	27	80%	30
S. aureus	60%	22	70%	24	80%	28

Table 2: Zone of inhibition of Ag NPs antibiotics against *E. coli* and *S. aureus*

Nanoparticles can attach to the membrane of bacteria by electrostatic interaction and disrupt the integrity of the bacterial membrane (25) The heightened reactivity of Ag NPs can be contributed to the increasing surface area compared with volume ratio (26,27,28). It was shown the inhibitory effect of silver nanoparticles to the high affinity of silver ions which interacts with the sulfur groups of the cysteine which is one of the main components of the cell membrane of the bacteria which leads to the denaturation of the bacteria (29). In conclusion, the inhibitory effect of silver nanoparticles can be attributed to the high affinity of silver ions which interacts with the sulfur groups of the cysteine which is one of the main components of the cell membrane of the bacteria which leads to the denaturation of the bacteria.

REFERENCES

[1] Wang, L., Hu, C., & Shao, L. (2017). The antimicrobial activity of nanoparticles: present situation and prospects for the future. *International journal of nanomedicine*, *12*, 1227.

[2] Godymchuk, A., Frolov, G., Gusev, A., Zakharova, O., Yunda, E., Kuznetsov, D., & Kolesnikov, E. (2015). Antibacterial properties of copper nanoparticle dispersions: influence of synthesis conditions and physicochemical characteristics. In *IOP Conference Series: Materials Science and Engineering* (Vol. 98, No. 1, p. 012033). IOP Publishing.

[3] Salem, W., Leitner, D. R., Zingl, F. G., Schratter, G., Prassl, R., Goessler, W., ... & Schild, S. (2015). Antibacterial activity of silver and zinc nanoparticles against Vibrio cholerae and enterotoxic Escherichia coli. *International Journal of Medical Microbiology*, *305*(1), 85-95.

[4] Ismail, R. A., Sulaiman, G. M., Abdulrahman, S. A., & Marzoog, T. R. (2015). Antibacterial activity of magnetic iron oxide nanoparticles synthesized by laser ablation in liquid. *Materials Science and Engineering: C*, *53*, 286-297.

[5] Kubacka, A., Diez, M. S., Rojo, D., Bargiela, R., Ciordia, S., Zapico, I., ... & Ferrer, M. (2014). Understanding the antimicrobial mechanism of TiO2-based nanocomposite films in a pathogenic bacterium. *Scientific reports*, *4*, 4134.

[6] Salem, W., Leitner, D. R., Zingl, F. G., Schratter, G., Prassl, R., Goessler, W., ... & Schild, S. (2015). Antibacterial activity of silver and zinc nanoparticles against Vibrio cholerae and enterotoxic Escherichia coli. *International Journal of Medical Microbiology*, *305*(1), 85-95.

[7] Pal S, Tak YK, Song JM. Does the antibacterial activity of silver nanoparticles depend on the shape of the nanoparticle? A study of the gram-negative bacterium Escherichia coli. Applied and environmental microbiology. 2007 Mar 15;73(6):1712-20.
[8] Theivasanthi T, Alagar M. Anti-bacterial Studies of Silver Nanoparticles. arXiv preprint arXiv:1101.0348. 2011 Jan 1.
[9] Abou El-Nour, Kh. M. M., Eftaiha, A., Al-Warthan, A., Amma, R. A. A., 2010.Synthesis and applications of silver nanoparticles. Arab. J. Chem. 3, 135–140.
[10] Yu, S. J., Yin, Y. G., Liu, J. F., 2013.Silver nanoparticles in the environment. Environ. Sci. Process. Impacts. 15 (1), 78–92.
[11] Prabhu and Poulose, 2012. Silver nanoparticles: mechanism of antimicrobial action, synthesis, medicalapplications, and toxicityeffects. Int. Nano Lett. 2, 32–39.
[12] Khot, L. R., Sankaran, S., Maja, J. M., Ehsani, R., Schuster, E. W., 2012. Applications of nanomaterials in agricultural production and crop protection: a review. Crop Prot. 35, 64–70.
[13] Simon, A.F., Daniels, R., Romero-Calderón, R., Grygoruk, A., Chang, H.Y., Najibi, R., Shamouelian, D., Salazar, E., Solomon, M., Ackerson, L.C., Maidment, N.T., Diantonio, A., Krantz, D.E. (2009). Drosophila vesicular monoamine transporter mutants can adapt to reduced or eliminated vesicular stores of dopamine and serotonin. *Genetics,181(2): 525—54.*
[14] Soenen, S.J; De Cuyper, M(2009).Intracellular iron oxide nanoparticle coating stability determines nanoparticle usability and cell functionality. *Contrast Media Mol Imaging*, 4; 207.
[15] Reidy B, Haase A, Luch A, Dawson KA, Lynch I. Mechanisms of silver nanoparticle release, transformation and toxicity: a critical review of current knowledge and recommendations for future studies and applications. Materials. 2013 Jun 5;6(6):2295-350.
[16] Tran QH, Le AT. Silver nanoparticles: synthesis, properties, toxicology, applications and perspectives. Advances in Natural Sciences: Nanoscience and Nanotechnology. 2013 May 14;4(3):033001.
[17] McShan D, Ray PC, Yu H. Molecular toxicity mechanism of nanosilver. Journal of food and drug analysis. 2014 Mar 31;22(1):116-27.
[18] Dakal TC, Kumar A, Majumdar RS, Yadav V. Mechanistic Basis of Antimicrobial Actions of Silver Nanoparticles. Frontiers in Microbiology. 2016;7.
[19] Rai M, Yadav A, Gade A. Silver nanoparticles as a new generation of antimicrobials. Biotechnology advances. 2009 Feb 28;27(1):76-83. Prabhu S, Poulose EK. Silver nanoparticles: mechanism of antimicrobial action, synthesis, medical applications, and toxicity effects. International Nano Letters. 2012 Dec 1;2(1):32.
[20] Xiu ZM, Zhang QB, Puppala HL, Colvin VL, Alvarez PJ. Negligible particle-specific antibacterial activity of silver nanoparticles. Nano letters. 2012 Jul 9;12(8):4271-5.
[21] Musee,N;Florian,P; Gudrun, Z ; Tim, C(2011).the antibacterial effects of engineered nanomaterials: Implications for wastewater treatment plants.*Journal of Environmental Monitoring*,13(5):1164-83.
[22] Lee, S., Song, K. and Lee, B. (2010). Antibacterial activity of silver nanoparticles prepared by a chemical reduction method, *Korean Journal of Chemical Engineering*, 27: 688-692.
[23] Mavani K, Shah M. Synthesis of silver nanoparticles by using sodium borohydride as a reducing agent. Int. J. Eng. Res. Technol. 2013 Mar 26;2(3).
[24] Ostad, S. N., Dehnad, S., Nazari, Z. E., Fini, S. T., Mokhtari, N., Shakibaie, M., and Shahverdi, A. R. (2010). Cytotoxic activities of silver nanoparticles and silver ions in parent and tamoxifen-resistant T47D human breast cancer cells and their combination effects with tamoxifen against resistant cells.*Avicenna journal of medical biotechnology*, *2*(4), 187.
[25] Seil JT, Webster TJ. Antimicrobial applications of nanotechnology: methods and literature. Int J Nanomedicine. 2012 Jan 1;7:2767-81.
[26] Sun Y, Xia Y. Increased sensitivity of surface plasmon resonance of gold nanoshells compared to that of gold solid colloids in response to environmental changes. Analytical Chemistry. 2002 Oct 15;74(20):5297-305.
[27] Kittler S, Greulich C, Diendorf J, Koller M, Epple M. Toxicity of silver nanoparticles increases during storage because of slow dissolution under release of silver ions. Chemistry of Materials. 2010 Jul 30;22(16):4548-54.
[28] Ravishankar Rai V, Jamuna Bai A. Nanoparticles and their potential application as antimicrobials. Science against microbial pathogens: communicating current research and technological advances. Badajoz, Spain: Formatex Research Center. 2011:197-209.
[29] Singh M, Singh S, Prasad S, Gambhir IS. Nanotechnology in medicine and antibacterial effect of silver nanoparticles. Digest Journal of Nanomaterials and Biostructures. 2008 Sep 1;3(3):115-22.
[30] Sekar M, Rashid NA. Formulation, Evaluation and Antibacterial Properties of Herbal Ointment Containing Methanolic Extract of Clinacanthus nutans Leaves.

RESEARCH PAPER Journal of Al-Qasim Green University (JQGU)

A SIMPLE DNA EXTRACTION METHOD FROM ASPERGILLUS SPECIES

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ABSTRACT

This study provides an efficient and low-cost genomic DNA extraction technique from Aspergillus flavus isolates. DNA was extracted from isolated fungal cellular extracts by applying lysis buffer. Then, simple salting out chemical treatment was implemented to retrieve DNA out of other contaminating compounds. All the extracted genomic DNA specimens were evaluated by both quality as well as quantity. Furthermore, PCR has confirmed the validity of the extracted DNA for downstream applications of molecular biology. This method ensures simple and straightforward technique for low budget and mediocre laboratories.

INTRODUCTION

The development of PCR-based molecular techniques has become one of the methods of choice for identification & characterization of fungi. It becomes important to isolate genomic DNA (gDNA) of superior quality and quantity for analyzing through PCR based applications [1]. Extraction of large quantity and high-quality DNA is often a limiting factor in the genetic analysis of the main important fungal isolates to agriculture. Many DNA extraction methods were available for extracting DNA from many fungal species. But, each of which suffers from a particular problem that made it non-competent enough to purify the DNA for several reasons [2]. Therefore, many companies around the world were provided many solutions to these problems by providing highly specific kits for genomic DNA (g DNA) extraction, such as that relies on Polyvinyl polypyrrolidone (PVPP), Sephadex G 200, Q-Sepharose, electroelution, and silica gel. Nevertheless, the coherent high-cost problem still represents the main obstacle in many large scale DNA isolation purposes [3]. This problem is maximized in the limited budget laboratories. Alternatively, several trials were made to provide a low cost – efficient technique for DNA extraction for many fungal species [4],[5]. However, many utilized techniques were relied on several relatively high-cost enzymes in their procedures and therefore render the cost of these techniques comparable to other commercially available kits. Nonetheless, there were some innovated DNA extraction protocols that extract fungal gDNA without using any enzymatic treatment [6]. But, however, the absence of all enzymes may however, reduce the overall efficiency of these techniques. Therefore, this study was designed to conquer all the previously mentioned problems by presenting a low cost – efficient alternative method that can isolated fungal gDNA with high competency and reliability.

MATERIALS AND METHODS

Collection of samples

A. flavus isolates were isolated from rice following the Food and Agricultural Organization (FAO) standard methods [7]. A total of one hundred Iraqi rice samples including were isolated randomly from local markets. The initial amount of the collected sample of rice grains was about 3 Kg. The samples were then divided through a grain precision divider to obtain about 100 g of each sample. Then, five grains of each divided

sample were placed randomly in 9 cm diameter Petri dishes containing potato dextrose agar (PDA) medium. The Petri-dishes were incubated at 25±2 ˚C and observed daily for the emergence of colonies for five days, after which the observed colonies were counted. All the fungal isolates were identified up to the species level and their morphological characteristics of fungal isolates were identified using keys and manuals [8]. Out of other observed microorganisms,

DNA extraction

Briefly ,inoculated of each isolates and incubated for 7 days at 25 c^{o} then 10 ml centrifuge tube is filled with 5 ml of liquid Potato-dextrose medium (Fig. 1, step 1). The mycelial mat is pelleted by centrifugation for 5 minutes at 6000 rpm in a centrifuge (EBA 20, Hettich, 32278 Kirchlengern, Germany) (Fig. 1, step 2). The mycelial mat is washed with 5 ml of TE (10 mM Tris-HCl, 1mM EDTA, pH 8.0) buffer and pelleted again (Fig. 1, step 3). The TE buffer is decanted and 3 ml of extraction buffer (0.25 M NaCl, 0.025 M EDTA, 0.5% SDS, 0.2 M Tris-HCl, pH 8.5) is added (Fig. 1, step 4). The mycelium is crushed manually with a compatible glass rod and for about three minutes or more (Fig. 1, step 5). To remove RNA contamination, 0.5 ml of RNase A solution (10 mg/ml) is added (Favrogen Biotech Co., Changzhi Township, Taiwan), and incubated at 37˚C for 30 min (Fig. 1, step 6). Then, a 1.5 ml of 3 M sodium acetate, pH 5.2 is added, and the tubes are placed at -20°C for ten minutes (Fig. 1, step 7). Tubes are then centrifuged and the supernatant transferred to 1.5 microcentrifuge tube (Fig. 1, steps 8 - 9). An equal volume of isopropanol is added and incubated at least 5 minutes at room temperature (Fig. 1, step 10). The precipitated DNA is pelleted by centrifugation at maximum speed a microfuge (Prism R, Labnet, USA) (Fig. 1, steps 11 - 12). The supernatant is discarded while the pellet is washed with 70% ethanol, mixed well, and centrifuged again at maximum speed in a microfuge (Fig. 1, step 13 - 14). The pellet is air-dried for several minutes and re-suspended in 50 µl of TE buffer (Fig. 1, step 15).

DNA quantification

The concentration and purity of DNA were measured by a nanodrop (BioDropµLITE, BioDrop Co., UK) (Fig. 1, step 16). Besides, the purity of DNA samples was measured automatically by calculating ration 260/280.

PCR design and amplification

To give further indication of the purity of the extracted DNA, one PCR fragment was selected for amplification. The sequence of forward primers was 5'-gtggacggacctagtccgacatcac-3' and the sequence of the reverse primer were 5'-gtcggcgccacgcactgggttgggg-3' [9]. The lyophilized primers were purchased from Bioneer (Bioneer, daejeon, South Korea). The pcr reaction was performed using accupower pcr premix (Bioneer). Each 20µl of pcr premix was contained 1 u of top dna polymerase, 250 µm of dntps, 10 mm of tris-HCl (ph 9.0), 30 mm of KCl, 1.5 mm of mgcl2. The reaction mixture was completed with 10 pmol of each primer and 50 ng of genomic dna. The following program was applied in pcr thermocycler (mastercycler-nexus, eppendorf). the amplification was begun by initial denaturation at 95°c for 5 min, followed by 30 cycles of denaturation (95°c, 1 min), annealing (65 °c, 1min), and elongation (72 °c, 1 min), and was finalized with a final extension at 72°c for 5 min. amplification was verified by electrophoresis in 1.5% (w/v) agarose gel in 1× TBE buffer (2 mm of edta, 90 mm of tris–borate, ph 8.3).

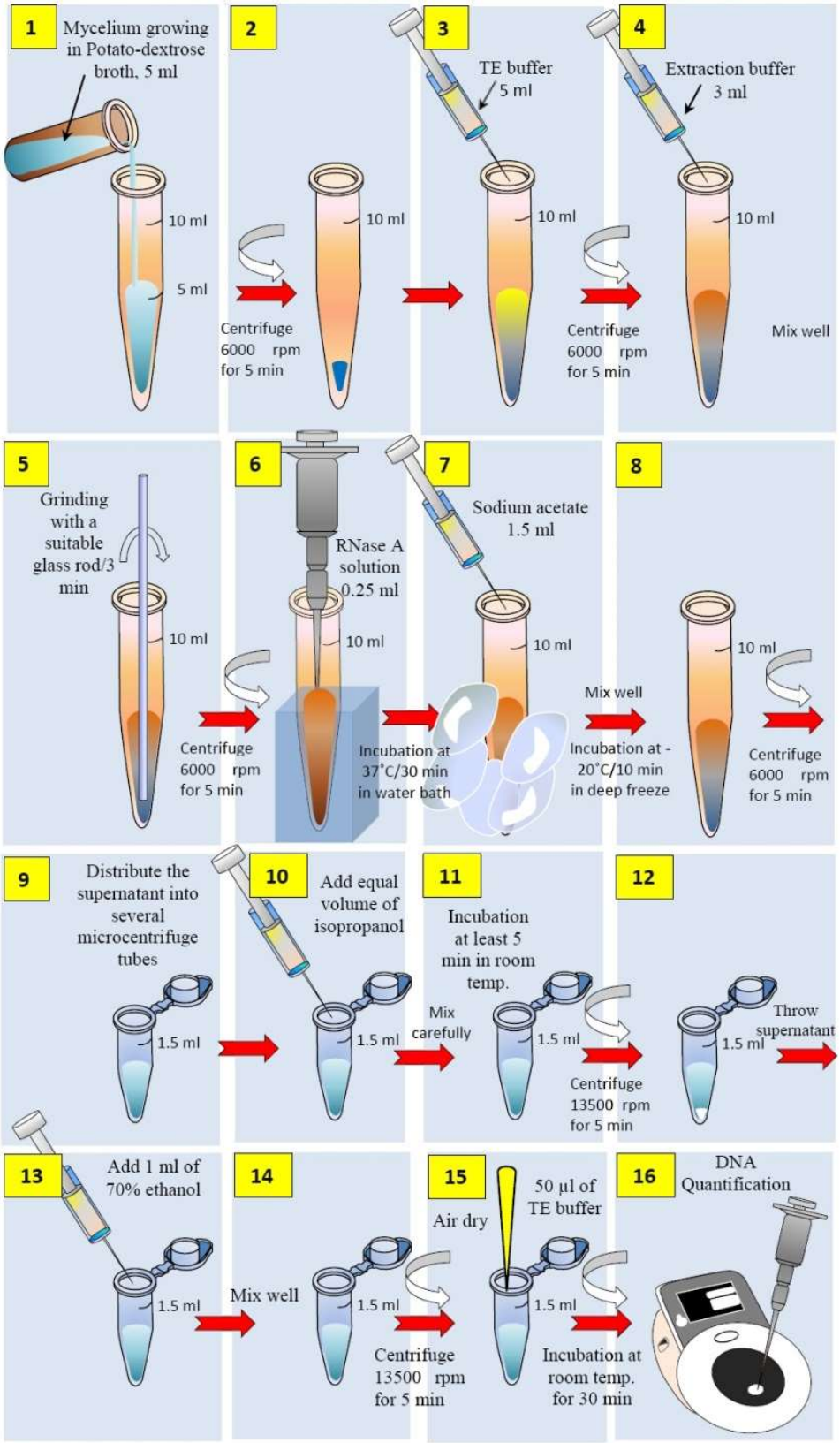

Figure 1, (steps 1 - 16). A schematic diagram for the main DNA extraction steps used in the presented DNA extraction method from the native fungal isolates. Then, the DNA integrity was checked by a standard 0.8% (w/v) agarose gel electrophoresis that is pre-stained with a higher concentration of ethidium bromide (0.7 µg/ml) in TAE (40 mM Tris acetate; 2 mM EDTA, pH 8.3) buffer, using a 1 kb ladder as a molecular weight marker (Cat # D-1040, Bioneer, Daejeon, South Korea). Agarose gel was visualized in a UV transilluminator provided with gel documentation unit (Chemidoc/Bio-Rad – USA). The isolated DNA was used as a template for PCR as shown.

RESULTS AND DISCUSSION

In the present study, a new modification of Cenis method for DNA extraction was presented. Though Cenis method was can rely on many published data we have shown that this method suffers from several limitations in terms of the presence of RNA contamination adhere with the extracted genomic DNA (Cenis 1992). Besides, Cenis method utilized specialized equipment for lysing fungal cell walls. Therefore, our improvised method provides a solution to the main difficulties in the Cenis manual method. However, the high quality and the adequate quantity of the extracted DNA was confirmed using Nanodrop technique (Table 1). It was found that this method has a reasonable quality and acceptable quantity all over the samples. However, the present study has shown that the average quality of the extracted gDNA was ranged around 1.6 to 1.9, which represents the main acceptable ratio of the highly qualified gDNA. Add to that, this method has provided adequate amounts of gDNA that extended from 13 µg/ml into 96 µg/ml. however, these amounts were highly satisfied since only about 0.1 µg is enough to perform the PCR reaction [10]. After the preliminary confirmation of the quality and the quantity of the gDNA, it is very necessary to provide a positive indicator about the suitability of this gDNA into the downstream applications of molecular biology experiments, such as PCR. Therefore, a further indication was provided by performing a direct conventional PCR. PCR, in turns, has indicated the eligibility of this gDNA to be used as a successful template for these experiments (Figure 2). The electrophoresed PCR amplicons were shown highly specific bands for almost all amplified omt1 locus from all 25 A. flavus isolates. The very clear and highly dense DNA bands that were readily visualized were indicated the feasibility of this simple and straightforward DNA extraction technique to be used in the wider spectrum for more fungal species. In conclusion, this study suggests that this simplified presented technique is a good and low-cost alternative for genomic DNA isolation for PCR experiments. This method was only applied on Aspergillus species and it is highly recommended to broaden the applicability of this DNA extraction technique on further fungal species.

Table 1: Nanodrop measurement of the quality (µg/ml) and the quantity (260/10 reads) of the extracted genomic DNA from *Aspergillus flavus* isolates.

No.	Quantity	Quality
1	28.96	1.7
2	28.28	1.6
3	14.42	1.4
4	14.75	1.7
5	10.36	1.4
6	96.14	1.8
7	18.56	1.9
8	17.75	1.5
9	13.16	1.8
10	57.85	1.6
11	15.45	1.6
12	36.49	1.7
13	26.29	1.6
14	31.30	1.8
15	10.70	1.6
16	32.88	1.8
17	31.50	1.8
18	14.13	1.8
19	84.05	1.7
20	13.93	1.4
21	31.37	1.7
22	47.37	1.8
23	197.9	1.6
24	34.92	1.8
25	30.07	1.8

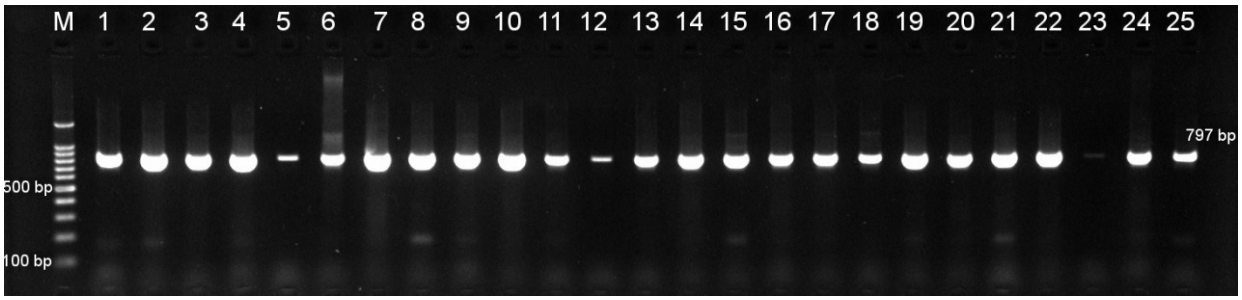

Figure 2. confirmative PCR for the genomci DNA that was extracted from 25 Aspergillus flavus native isolates using a simple modified method. The product lengths was 797 bp. M refers to ladder marker, while numbers 1 to 25 refer to PCR products results from 25 Aspergillus flavus isolates respectively.

REFERENCES

[1] Aamir S, Sutar S, Singh SK, Baghela A 2015 – A rapid and efficient method of fungal genomic DNA extraction, suitable for PCR based molecular methods. Plant Pathology & Quarantine 5(2), 74–81.
[2] Fredricks DN, Smith C, Meier A. Comparison of Six DNA Extraction Methods for Recovery of Fungal DNA as Assessed by Quantitative PCR. Journal of Clinical Microbiology. 2005;43(10):5122-5128.
[3] Devi SG, Fathima AA, Radha S, Arunraj R, Curtis WR, Ramya M (2015) A Rapid and Economical Method for Efficient DNA Extraction from Diverse Soils Suitable for Metagenomic Applications. PLoS ONE 10(7): e0132441.
[4] Liu D, Coloe S, Baird R, Pedersen J. (2000). Rapid mini-preparation of fungal DNA for PCR. J Clin Microbiol 38:471–471.
[5] Gontia-Mishra I., Tripathi N., Tiwari S. (2014). A simple and rapid DNA extraction protocol for filamentous fungi efficient for molecular studies. Indian Journal of Biotechnology 13, 536-239.
[6] Cenis, J.L., 1992. Rapid extraction of fungal DNA for PCR amplification. Nucl. Acids Res., 20(9): 2380.
[7] FAO, 1992. Manual of food quality control, 4 Rev., 1- Microbiological Analysis.
[8] Klich, M.A. Identification of Common Aspergillus Species, Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands, 2002.
[9] Giesen R. (1996). Multiplex Polymerase Chain Reaction for the Detection of Potential Aflatoxin and Sterigmatocystin Producing Fungi. System. Appl. Microbiol. 19, 388-392
[10] Sambrook, J. and Russell, D.W. (2001) Molecular cloning: a laboratory manual. Vol. 2, 3rd edn. Cold Spring Harbor Laboratory Press, New York.

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EFFECT OF FUNARIA SP. EXTRACTS ON FIRST STAGE OF CLUEX PIPIENS LARVA MOSQUITOES

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ABSTRACT

This study aimed to known the effect of aqueous and alcohol extracts of *Funaria sp.* on first stage of larva after 24 hours from exposure to different concentrations (0.5, 1.5, 2.5, 3.5, 4.5) mg/l of extracts. The results pointed that the percentage of mortalities for the first stage was (44, 59, 67, 78, 81) %, (29, 46, 54, 75, 75) %, and (61, 71, 79, 82, 93) %, and the value of LC_{50} was (2.1) mg/l, (0.75) mg/l, and (< 0.5) mg/l for cold water, hot water and alcohol extracts respectively. Also, results showed that alcohol extracts were more effective on first stage of larva than aqueous extracts, and the cold water extracts were also more effective on first stage of larva than hot water extracts. Results didn't point to a significant differentiation in level of probability 0.05, but showed that concentration have positive correlation coefficient with percentage of mortalities and negative correlation coefficient with percentage of survive.

Keywords: Larva, Extracts, LC_{50}, Moss, Mosquitoes

INTRODUCTION

Mosquitoes are most important group that belong to order Diptera that caused number of diseases to human and animals mostly in tropic places, where it caused illness that lead sometimes to the death such as Malaria, lymphatic filariasis, dengue haemorrhagic fever and Yellow fever [1]. *Culex pipiens* is mosquitoes' species that belong to group of culicidae family that sucked human and animals blood. These family could be transported disease and have fourth genera including *Culex*, these genus in many studies act as vectors of filariasis and some viral diseases [2]. The mosquitoes generally have ability to develop resistance to different chemicals after one generation [3] and produced many genes resistance that gave metabolic resistance [4] to the mosquitoes.

Funaria sp. is common type of water mosses that growth on moisture, shadow and wastes soil where the moisture was found and also can be found in place that has been destroyed by fires. Its genus belongs to order Funariales of the Plantae kingdom that spreading naturally in tropical and temperate zones [5]. Its known as green mosses or dense mats on the soil that have many effective compounds and remarkable unique substances with high biological activity [6]. Because of *Funaria sp.* belong to Bryophytes phylum for that it has important location in environment as food for some organisms and fuel for human also as decomposers for the rocks and protectors from soil erosion [7]. In *Funaria* the essential body of the plant is gametophyte (1n) that has leaves and stem and found in two forms. The first is protonema (juvenile) that has green epiterranean chloronemal branches which produced green parts above ground and non-green subterranean rhizoidal branches which produced semi root inside the soil [8].

This study aimed to know the effect of cold water, hot water and alcohol extracts of green parts of *Funaria sp*. on first stage of *Culex pipiens* larva. As for two reasons, first, because these stage more sensitive to any changes that could be happened in water environment where it lives. Second, to know if could be controlled or prevented the reproduction of *Culex pipiens* mosquitoes by controlled on first stage of larva.

MATERIALS AND METHODS

PREPARATION OF EXTRACTS

Same method of Ogundana *et.al.* (2016) and the way followed by Srivastava (2015) have been used. The green parts of mosses that growth above ground have been packed and washed with running fresh water to remove dust, dried at room temperature then powdered by electrical mill and sieved through the clean passage gauze to remove dried parts that didn't grind well. For preparing aqueous extracts, the weight of 10 gm of the preparing powder has been mixed well with 50 ml for each cold and hot distilled water separately. For preparing alcohol extract, also 10 gm from powder has been mixed well with 50 ml ethanol alcohol 70 %. Each one of extracts blended by an electric mixer and separated first by normal nomination papers and second by the centrifuge on speed of 3000 r / min for 15 minutes to have the filtrate. After that, the liquid filtrates were left to dry in room temperature to get a vegetable powder for each one of extracts. Then 0.5 gm from vegetable powder for three types of extracts has been mixed separate with 500 ml of distilled water to have 0.1 % concentration as stock solution to prepare (0.5, 1.5, 2.5, 3.5, 4.5) mg/l concentrations to each one of extracts. Finally, the extracts sterilized through special filter paper have 0.45 µm diameter holes and transferred to sterile bottles under refrigeration until the test.

BREEDING OF INSECT

Egg rafts of *C. pipiens* mosquitos has been collected through period from 1-8-2017 to 1-2-2018 from some stood water pools that found in the province of Babylon and translocated to the laboratory in plastic glasses that filled with water. The eggs stored for 1-2 days before using then transformed to the glass cup filled with water to add diet that composed from yeast and bran flour.

The way of El-Bokl (2016) and the method of Kitvatananchi *et.al.* (2005) have been used for preparing and breeding larvae in $28\pm1C^0$ temperature. The insect has been diagnosed in natural history museum in Baghdad university. For studying the effect of aqueous and alcohol extracts of green parts of *Funaria sp.* on first stage of larva; 30 larvae from first stage of larva that have less 24 hours of life have been transformed to another different glass cups 250 ml that filled separately with different concentrations for each one of extracts.

THE CALCULATION OF LC_{50}

To study the effect of cold water, hot water and alcohol extracts of green parts of *Funaria sp.* on *Culex pipiens* larva mosquitoes, the LC_{50} were found after using series of concentrations between (0.5-4.5) mg/l, in addition to control sample. After 24 hours from a cute exposure to different concentrations of each one of extracts, the average numbers of survive larva comparing with control sample have been calculated, then the percentage of mortalities and the lethal concentration for median (half) (LC_{50}) have been found by using the equation of straight line [Y= bx + a (a= intercept, b= slope)] [12] after the data corrected with abbott equation [13].

EXPERIMENTAL DESIGN AND STATISTICAL ANALYSIS

A completely randomized design (CRD) was used. Data were analyzed statistically by using less significant differences (LSD) at 0.05 after subjection to the analysis of variance and also find correlation coefficients between the results [14].

RESULTS AND DISCUSSION

The results in table (1) average of survival and percentage of mortalities of first stage of *Culex pipiens* larva after exposure to different concentrations of aqueous and alcoholic extracts showed that percentage of mortalities were increased with increasing concentration (0.5, 1.5, 2.5, 3.5, 4.5) mg/l with positive correlation coefficient, where the percentage of mortalities increases with increasing concentrations. Which the percentage of mortalities was (44, 59, 67, 78, 81) %, (29, 46, 54, 75, 75) %, (61, 71, 79, 82, 93) % for cold water, hot water and alcohol extracts respectively.

Table (1). Average of survival first stage of larva of *Culex pipiens* and percentage of mortalities after 24 hours from exposure to different concentration of green parts of *Funaria sp.* (30) (LSD= not found in results)

Concen. mg/l	Percentage of mortalities								
	Cold water extract			Hot water extract			Alcohol extract		
	Average of Survival	Percentage of survival	Percentage of mortalities %	Average of Survival	Percentage of survival %	Percentage of mortalities %	Average of Survival	Percentage of survival %	Percentage of mortalities %
0	27	90	10	28	93	7	28	93	7
0.5	15	56	44	20	71	29	11	39	61
1.5	11	41	59	15	54	46	8	29	71
2.5	9	33	67	13	46	54	6	21	79
3.5	6	22	78	7	25	75	5	18	82
4.5	5	19	81	7	25	75	2	7	93

This observation means the aqueous and alcoholic extracts of green parts of *Funaria sp.* have active materials against first stage of *Culex pipiens* larva that could be increased mortalities with increasing concentration in extracts. Also, results didn't appear a significant differentiation, which that could be returned to the same effective of different materials and compounds that effected on first stage of larva when extracted by different solvents. Also, many studies showed to find huge substances in plants, algae and mosses in different concentrations and needs diversity in solvent, pressure and temperature to extracts [15].

Results in figure (1, 2) appeared that cold water extracts were more effective than hot water extracts. Which the percentage of mortalities for cold water extracts was (44, 59, 67, 78, 81) % with average of survival (15, 11, 9, 6, 5) larva/30 larva, while the percentage of mortalities for hot water extracts was (29, 46, 54, 75, 75) % with average of survival (20, 15, 13, 7, 7) larva/30 larva, which could be referred to capability of aqueous extracts to extract compounds have high activity on first stage of *Culex pipiens* larva [16], or the aqueous extracts have varieties oxidation materials that could be interfered with iron metabolism [17] that important for innate immunity, or interfered with respiratory activities [18] of larva. The results also showed that value of LC_{50} (0.75) mg/l was lower for cold water extracts compared with value of LC_{50} (2.1) mg/l for hot water extracts, these different could be reason of destroyed some of these effective compounds by heating [19]. Or some of compounds couldn't extracted by hot water [20], or the high temperature of water makes the materials reaction between them to produce inert or semi active material [15] that couldn't passage through body of the larva. Generally, some studies pointed to find materials like alkaloids, flavonoids, steroids, phenols and saponins in cold water extract in high amount and few in hot water extracts that have toxicity on some organisms [21].

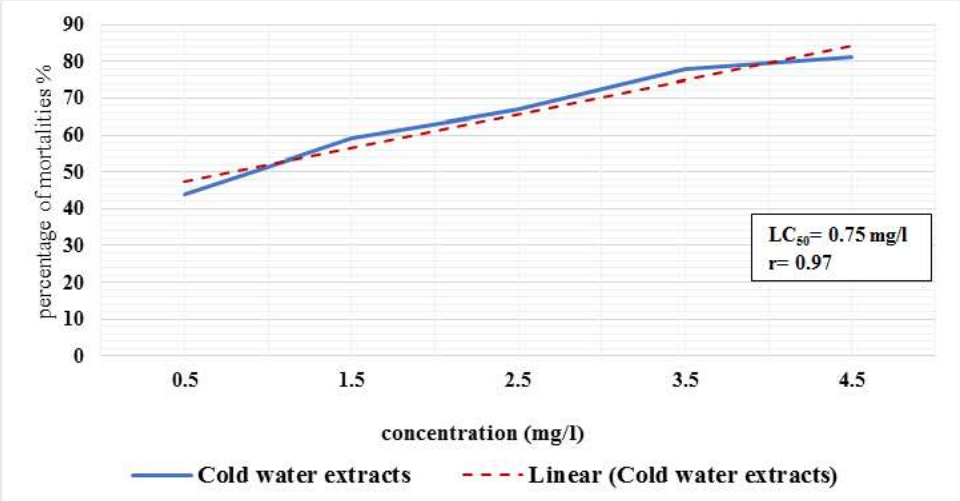

Figure (1). The value of LC_{50} for first stage of *Culex pipiens* larva after 24 hours of exposure to different concentration of cold water extracts of green parts of *Funaria sp*.

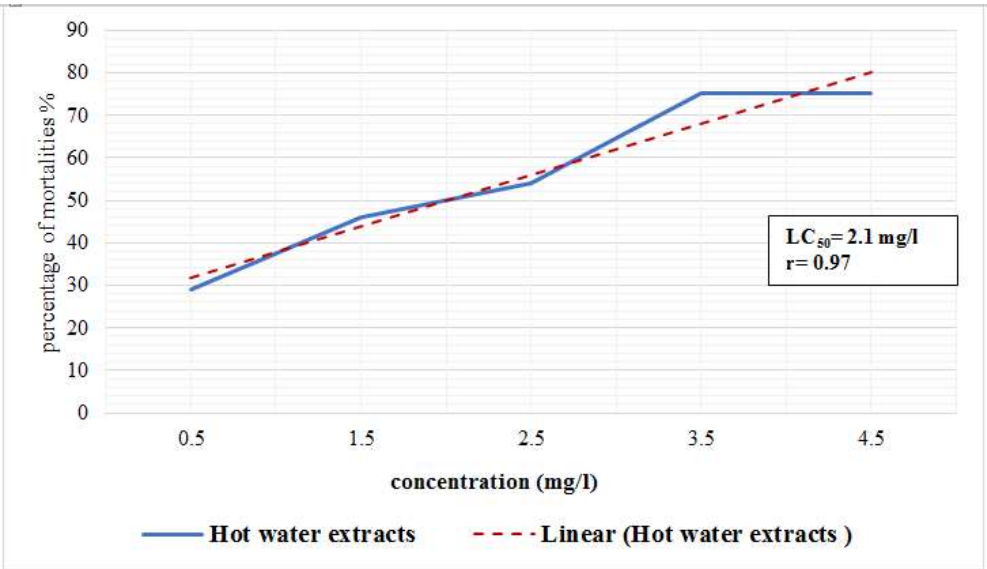

Figure (2). The value of LC_{50} for first stage of *Culex pipiens* larva after 24 hours of exposure to different concentration of hot water extracts of green parts of *Funaria sp*.

The results in Table (1) and figure (3) showed that alcohol extracts were most effective on first stage of larva than aqueous extracts. Which the percentage of mortalities was (61, 71, 79, 82, 93) % in average of survive (11, 8, 6, 5, 2) larva/30 larva with LC_{50} was (< 0.5) mg/l. The higher percentage of mortalities compare with aqueous extracts could be returned to content alcohol extract on most effective and toxic compounds such as essential oils [22], or some compounds that played roles as antimicrobials such as biflavonoids, hydroxy flavonoids, hypnogenols, dihydroflavonols polycyclic aromatic hydrocarbon and bioflavonoids [23].

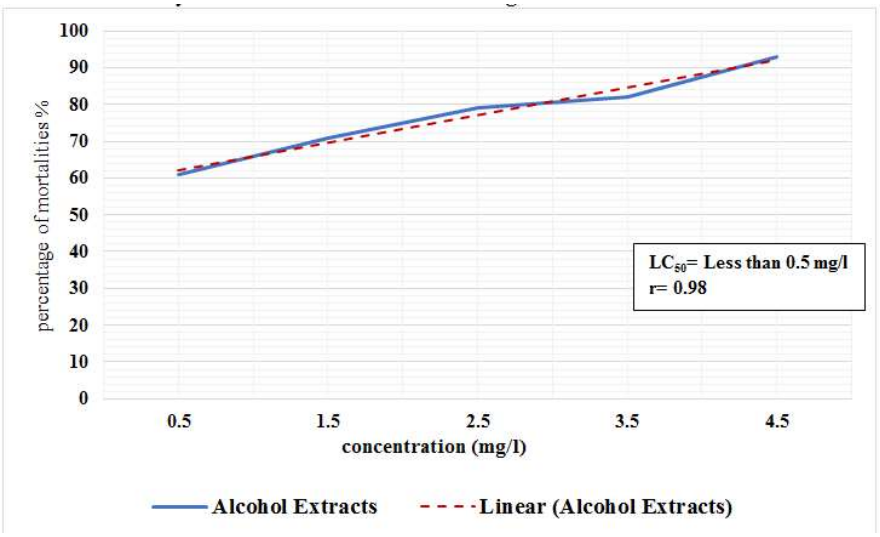

Figure (3). The value of LC_{50} for first stage of *Culex pipiens* larva after 24 hours of exposure to different concentration of alcohol extracts of green parts of *Funaria sp*.

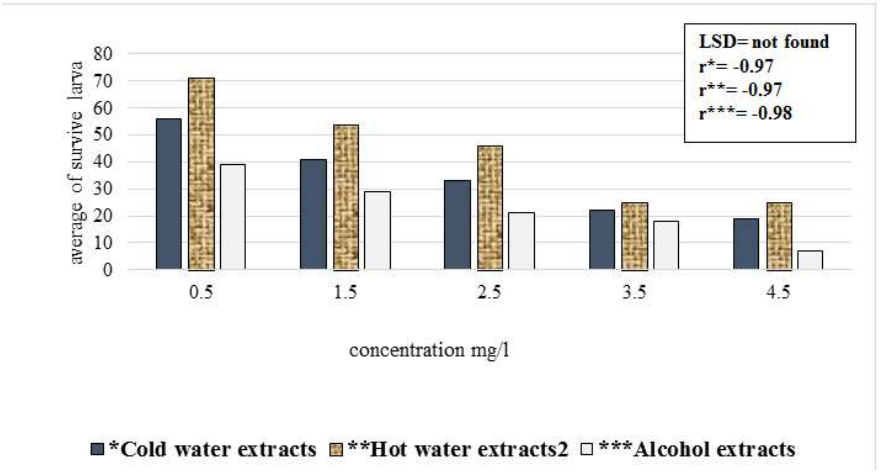

Figure (4). The percentage of survival for first stage of *Culex pipiens* larva after 24 hours of exposure to different concentration of aqueous and alcohol extracts of green parts of *Funaria sp*.

Or extraction higher amount of different chemicals that have ability to paralyze nerve system of larva [24]. Also, the lower value of LC_{50} referred to higher activity of alcohol extracts components on first stage of larva. Which many studies pointed to alcohol extracts as a solvent that have potential larvicidal activity especially nonpolar components in addition to polar compounds [11] that have ability to confuse endocrine system [25], or dissolve uncompleted cuticle that cover larva body [26], or disturbed nervous and hormonic system [27] that lead eventually to delay the growth or dead the larva. In conclusion, the results showed that aqueous and alcohol extracts of *Funaria sp.* can be caused the mortalities to the first stage of *Culex pipiens* larva and positive correlation coefficient with percentage of mortalities and negative correlation coefficient with percentage of survive.

REFERENCES

1- Jupp, P. G. (2004). Vectors: mosquitoes in 'Infectious diseases of livestock' Vol.1., Editors Coetzer, J. A. W. and Tustin, R. C., 2nd Edition, Oxford University Press.
2- Silver, J. B. (2008), Mosquito Ecology –Field sampling methods, Springer, 3rd Edition.
3- Labbe, P.; Berticat, C.; Berthomieu, A.; Unal, S.; Bernard, C.; Weill, M. and Lenormand, T. (2007). Forty years of erratic insecticide resistance evolution in the Mosquito *Culex pipiens*. PLoS Genetics, 3:2190–2199.
4- Fellous, S. and Lazzaro, B. P. (2010). Larval food quality affects adult (but not larval) immune gene expression independent of effects on general condition. Molecular Ecology 19:1462–1468.
5- Asakawa, Y.; Ludwiczuk, A. and Nagashima, F. (2013). Chemical Constituents of Bryophytes: Bio- and Chemical Diversity, Biological Activity, and Chemosystematics (Progress in the Chemistry of Organic Natural Products); Springer: Vienna, Austria, p: 796.
6- Klavina, L.; Springe, G.; Nikolajeva, V.; Martsinkevich, I. *et.al.,* (2015). Chemical Composition Analysis, Antimicrobial Activity and Cytotoxicity Screening of Moss Extracts (Moss Phytochemistry). Molecules, 20: 17221-17243.
7- Stark, L. R.; Greenwood, J. L.; Brinda, J. C. and Oliver, M. J. (2013). The desert moss *Pterygoneurum lamellatum* (Pottiaceae) exhibits an inducible ecological strategy of desiccation tolerance: Effects of rate of drying on shoot damage and regeneration. American Journal of Botany, 100: 1522-1531.
8- Banu-Fattah, K. (2005). *Funaria hygrometrica* hedw. (Funariaceae) from Bangladesh. Bangl. J. Bot. 34(2): 121-124.
9- Ogundana, E. I.; Adelanwa, M. A.; Adamu, A. K. and Ella, E. E. (2016). Antimicrobial effects of *Gomphrena Celosioides* extracts on *Staphylococcus* species isolated from women with vulvovaginitis in Zaria, Nigeria. Ann. of Experim. Biol., 4 (1): 7-12.
10- Srivastava, N. (2015). *Funaria Hygrometrica* extracts with activity against plant pathogenic fungi *Alternaria species.* International Journal for Environmental Rehabilitation and Conservation, 1 (2): 105 – 112.
11- El-Bokl, M. M. (2016). Toxicity and bioefficacy of selected plant extracts against the mosquito vector *Culex pipiens* L. (Diptera: Culicidae). Journal of Entomology and Zoology Studies, 4(2): 483-488.
12- Kitvatanachai, S.; Apiwathnasorn, C.; Leemingsawat S.; Wongwit, W. and Overgaard, H. J. (2005). Determination of lead toxicity in *Culex quinquefasciatus* mosquitoes in the laboratory. Sou. ea. Asi. J. of Trop. Medi. Pu. Heal., 36 (4).
13- Abbott, W. S. (1925). A method of computing the effectiveness of an insecticide. J. Econ. Entomol., 18: 265–267.
14- Devore, J.; Farnum. N. and Doi, J. (2014). Applied Statistics for Engineers and Scientists. Cengage Learning, third edition, San Luis Obispo, California Polytechnic State University, Printed in the United States of America, pp: 656.
15- Gil-Chávez, G. J.; Villa, J. A.; Ayala-Zavala, J. and *et.al.,* (2013). Technologies for Extraction and Production of Bioactive Compounds to be Used as Nutraceuticals and Food Ingredients: An Overview. Comprehensive Reviews in Food Science and Food Safety, 12: 1-19.
16- Nikolajeva, V.; Liepina, L.; Petrina, Z.; Krumina, G.; Grube, M. and Muiznieks, I. (2012). Antibacterial Activity of Extracts from Some Bryophytes. Advances in Microbiology, 2, 345-353.
17- Yun, E. Y., J. K.; Lee, O. Y.; Kwon, J. S.; Hwang, I.; Kim, S. W.; Kang, W. J., Lee *et al.* (2009). *Bombyx mori* transferrin: genomic structure, expression and antimicrobial activity of recombinant protein. Developmental and Comparative Immunology, 33:1064–1069.
18- Thareja, V.; James, A.; Singh, R.; Naorem, A. S. *et.al.,* (2017). Overwintering sites and strategies of *Culex quinquefasciatus* (Diptera: Culicidae) larvae and impact of neem seed kernel aqueous suspension (NSKAS) and other eco-friendly tools on them. International Journal of Mosquito Research, 4 (2):135-141.
19- Hartonen, K.; Kronholm, J. and Riekkola, M. L. (2005). Utilisation of high temperature water in the purification of water and soil. Laboratory of Analytical Chemistry, Department of Chemistry, University of Helsinki, ISBN 952-10-2817-3.
20- Abd El-Azim, W. M.; Khater, R. M. R. and Badawy, M. Y. M. (2017). Effect of Bio-Fertilization and Different Licorice Extracts on Growth and Productivity of *Foeniculum vulgare*, Mill. Plant. Middle East Journal of Agriculture Research, 6 (1) :01-12.
21- Adebiyi, A. O.; Oyedeji, A. A.; Chikwendu, E. E. and Fatoke, O. A. (2012). Phytochemical Screening of Two Tropical Moss Plants: *Thidium gratum* P. Beauv and *Barbula indica* Brid Grown in Southwestern Ecological Zone of Nigeria. Amer. J. of Anal. Chem., 3: 836-839.
22- Üçüncü O.; Cansu T. B.; Özdemir T.; Karaoğlu, A. Ş. and Yayli, N. (2010). Chemical composition and antimicrobial activity of the essential oils of mosses (*Tortula muralis* Hedw., *Homalothecium lutescens* (Hedw.) H. Rob., *Hypnum cupressiforme* Hedw., and *Pohlia nutans* (Hedw.) Lindb.) from Turkey. *Turk. J. Chem.* 34: 825–834.
23- Veljić M, Tarbuk M, Marin PD, Ćirić A, Soković M, Marin M. (2008). Antimicrobial activity of methanol extracts of some genuine mosses from Serbia. Pharmac. Biol., 46 (12): 871-875.
24- Vézilier, J.; Nicot, A.; Lorgeril, A. D.; Gandon, S. and Rivero, A. (2013). The impact of insecticide resistance on *Culex pipiens* immunity. Evolutionary Applications, 6: 497–509.

25- Gulia-Nuss, M.; Robertson, A. E.; Brown, M. R. and Strand, M. R. (2011). Insulin like peptides and the target of rapamycin pathway coordinately regulate blood digestion and egg maturation in the mosquito *Aedes aegypti*. PLOS ONE, doi:101371/journalpone0020401.

26- Alkhalaf, A. A. (2013). Ultrastructural Effects of *Matricharia chamomella* Extracts on Cuticle of 3rd Larval Instar of *Culex quinquiefasciatus*, Journal of Mosquito Research, 3(10): 71-75.

27- Sharma, P.; Mohan, L. and Srivastava, C. N. (2006). Phytoextraction induced developmental deformities in malaria vector. Bioresource Tech, 97:1599-604.

28- Preet, S. and Gupta, S. (2017). Genetic diversity studies on *Culex quinquefasciatus* from diverse larval habitats using RAPD-PCR marker. International Journal of Mosquito Research, 4(2): 93-98.

29- Pushpalatha E. (2015). Acute Toxicity of Two Tropical Plant Extracts on the Fecundity and Fertility of *Culex quinquefasciatus* Say. Advances in Zoology and Botany, 3(3): 38-41.

30- Jeong, S. C.; Tulasi, R. and Koyyalamudi, S. R. (2016). Antioxidant Capacities of Hot Water Extracts and Endopolysaccharides of Selected Chinese Medicinal Fruits. J. of Cancer, 8 (33): 1-13.

31- Tseng, Y. H. and Mau, J. L. (2007). Antioxidant properties of cold water extracts from *Ganoderma tsugae*. Fung. Sci. 22(1, 2): 13–23.

EFFECT OF INJECTION OF HATCHING EGGS IN DIFFERENT CONCENTRATIONS NANO SILVER AT AGE 17.5 DAYS IN SOME OF THE PRODUCTIVE CHARACTERISTICS OF BROILERS ROSS 308 EXPOSED TO HEAT STRESS

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ABSTRACT

This study was conducted in the poultry farm of the Department of Animal Production - Faculty of Agriculture / AL-Qasim green University and for the period from 19/8/2017 until 22/9/2017. The laboratory work was then carried out. 480 chicken broilers and Cages 1 × 1.5 m The chicks were randomly divided into six treatments and each treated four replicates, each containing 20 chicks, nano-silver was injected into the eggs at concentrations (0, NaCl, 12ppm, 14ppm, 16ppm, 18ppm) for (T1, T2, T3, T4, T5 and T6) respectively, and raised the chicks in the farm for 35 days and in this study we found the following results. Significant increase (P <0.01) in live body weight at week 5 and total weight gain for weeks (1-5) of bird age compared with negative control coefficients T1 and positive control T2, A significant improvement (P <0.01) was observed in the total feed consumption of the birds of the nano-silver injection treatments compared to the negative and positive control coefficients and There was a significant superiority (P <0.05) in the overall dietary conversion efficiency of all injection treatments compared to the negative control treatment T1and there was not significant in biological percentage(%) and total percentage of depreciation(%) .

Keywords: nano-silver, injection, broiler

INTRODUCTION

Nano-technology is a new methodology based on the use of nanoparticles as potential vehicles to transport nutrients to specific targets, thereby improving their bioavailability, silver belongs to the group of noble metals and silver nanoparticles have affinity for primary amines (protein N-termini and Lys side chains), exhibit tropism for connective tissue and also have anti-microbial properties Thus, they may be used as particles that can transport amino acids to tissues and their cells, It has also been reported that silver nanoparticles can kill microbes more rapidly and thoroughly than their cationic forms [8]. The significance of using nano-particles is due to the dramatic increase in surface area in relation to their size. Moreover, their nano-scale (1–100 nm) also allows them to pass through biological barriers (e.g. to the brain and eye); however, nano-particle bio-distribution cannot be predicted once the solution has entered the body, which can be affected by reactions with proteins and the mononuclear phagocyte system [18], The researcher pointed out [10]that injecting eggs hatching in silver nano-particles had no effect on growth performance or the histological picture of the jejunum. An increase was noted in the total number of aerobic mesophilic bacteria and a decrease in the number of coli group bacteria, which are facultative anaerobes, which indicates that the nano-silver had a selective effect on the microflora of the digestive tract in the chickens. Also, The researcher [20] concluded that Nano silver either alone or in combination with amino acids (Cysteine, threonine) did not affect embryonic growth but improved immune competence, indicating that nano-silver and amino acid complexes can act as potential

agents for the enhancement of innate and adaptive immunity in chicken. That the rise of environmental temperatures is an important problem in the production of poultry in areas with a hot climate in the summer months, especially when combined with high humidity, which places severe pressure on the birds lead to low performance. Losses from heat in the United States were estimated at more than $ 1.7 billion in 2003 [19]. Statistical data for temperatures indicate a rise of 0.2 ° C per decade and this number is likely to increase in the future [21]. The current study aims to study the effect of injecting hatching eggs at different levels of nano-silver in the production characteristics of broiler chickens and to know which levels are better.

MATERIALS AND METHODS

The NaCl solution was used to prepare the egg injection solution, injected with nano-silver (0.25 ml/egg) and 20 nm, the solution density (10.5 g / cm3) and the morphological shape of the nano-silver particles (spherical) from Nanosany corporation .co.

The weight of the body and the increase in weight

The weight of the body of each treatment was calculated at the end of each week and for weeks (1 - 5) by weight of all refined birds. The live weight of the refined was calculated as follows [5]:

Live weight (g) =Total live weight of birds at the end of the week (g)/ Number of birds at the end of the week. The weekly increase rate (g / replicates) is as follows: Average body weight end of the week (gm) - Average body weight at the beginning of the week (gm).

Feed consumption

The weekly feed consumption rate for refined birds and for weeks (1 - 5) was calculated by weight of the feed provided at the beginning of the week minus the remaining feed weight at the end [5].

The efficiency of food conversion

The conversion efficiency of food was calculated according to the formula referred to [4] Food Conversion Efficiency Weekly=Average amount of feed consumed (gm) in the week/Average weight increase (gm) in the week

Statistical analysis

The Statistical Analysis System [16] used data analysis to study the effect of different coefficients in the studied traits in full randomized design (CRD). The differences between the averages were compared with the [6] multidimensional test. Mathematical Model: $Yij = \mu + Ti + eij$

Food treatment

The chicks were fed on the starter from day one until the third week of the bird's age. Then the finisher was replaced until the end of the fifth week. Feed and water were provided ad libitum and the feed used was as shown in the table 1.

Table 1. the percentage of the ingredients used in the study and their chemical composition

%The finisher	% The beginning	Feeding materials
40	30	yellow corn
24	28.25	Wheat
24.8	31.75	Soybeans (48% protein)
5	5	*Proteins concentrate
4.4	2.9	Sunflower oil
0.6	0.9	Limestone
0.9	0.7	(DCP) Calcium diphosphate
0.1	0.3	Nacl
0.2	0.2	mixture of vitamins and minerals
100	100	Total
** Calculated chemical analysis		
20	23	(%) General protein
3195.3	3027	(Representative energy calculated (kilo calories / kg feed
1.1	1.2	(%) Lysine
0.46	0.49	(%) Methionine
0.32	0.36	(%)Cyctain
0.76	0.85	(%) Cyctain + Methionine
0.49	0.45	(%) Phosphorus is available
159.77	131.61	% C/P

*Procium processor: brokon-5 special w: chinese origin, each kg contains 40% raw protein, 3.5% fat, 1% fiber, 6% calcium, 3% phosphorus available, 3.25% lysine, 3.90% methionine + 2.2% sodium, 2,100 kg / kg of energy represented, 20000 iu vitamin a, 40000 iu vitamin d3, 500 mg vitamin e, 30 mg vitamin k3, 15 mg vitamin b1 + b2, 150 mg b3, 20 mg b6, 300 mg b12, 10 mg folic acid, 100 µg biotin, 1 iron mine, 100 mg copper, 1.2 mg manganese, 800 mg zinc, 15 mg iodine, 2 mg selenium, 6 mg cobalt, 900 mg antiphosphate . **according to the chemical analysis of feeding according to nrc (1994).

RESULTS AND DISCUSSION

The rate of body weight and weight increase

The results of Table (2) Shows the effect of nano-silver in the body weight. The same table showed height significant (P <0.01) during the first week of the birds' age of T4 treatment on the rest of the treatments T1, T2, T3, T5, and T6, while were significant to T3 on T2, T6. T1 and T5 were not significantly different. The second week showed a significant difference (P <0.01) for T4 compared with the other treatments. T5 and T3 were superior to T6 on T2 and T1. The third week showed significantly (P <0.05) to T3, T4, T6 on others treatment and significantly to T1, T5 on T2 significantly higher (P <0.01) for T6, followed by T4 compared with treatment others in experimental forth week and significant to T5 on T1, T2, T3 and significant T3 on T1, T2 while no difference was between T1 and T2. In the fifth week were significantly higher (P <0.01) to T6 followed by T5, T4, and T2, superior to T3 on T1, where came the lowest in fifth week Wight (1944.78 g).

Table (3) shows the effect of injection of hatching eggs in nano-silver in the rate of increase of weight of different weeks (gm). It shows that there is a significant difference in the level of (P <0.01) between the different treatments in the rate of weight increase during the first week, where nate a significant treatment T4 followed by the treatment T3 respectively on the rest of the treatments T1, T2, T5, and T6 were also superior to T1 and T6 on T2, T5, T2, and T5 showed no difference between them. In the second week were also significantly higher (P <0.01) in T4, T5 followed by T3 compared with other treatment and significant T6 on T1, T2 also significant T2 on T1. In third week was significantly higher (P <0.01) to T6 on treatments T1, T2, T3, T4, T5 and significant T1 on T2, T3, T4, T5 also significant T4, T3, T5 respectively on T1.in forth week the table showed significantly higher (P <0.01) in T2 on other treatment T1,T3,T4,T5 and T6 and significant T5,T6 on T1,T3,T4 also significant T1 on T3,T4.whil in fifth week were note significantly higher (P <0.01) in T6 treatment compared other treatment and height significant in T5,T4,T3,T2 respectively compared with T1,the total increase in weight Table (3) showed significant superiority of the results (P <0.01) in treatment T6, T5, T4 respectively compared to treatment T3, T2, T1 while was found superior the positive control treatment T2 and T3 treatment on T1 Where its came the last treatment in increase of weight by weight (1902.78 gm).

Table 2. Effect of injection of hatching eggs in nano-silver in live body weight (gm) for different weeks of meat breeds exposed to heat stress.

Mean ± standard error (gm)					treatment
fifth week	fourth week	third week	second week	First week	
f 1.14 ± 1944.78	c 1.86 ± 1387.28	ab 0.49 ± 853.28	e 0.87 ± 453.26	d 0.46 ± 167.62	T1
d 2.95 ± 2014.73	c 1.71 ± 1387.08	b 2.44 ± 825.86	d 1.08 ± 457.13	e 0.25 ± 164.82	T2
e 1.32 ± 2002.27	d 0.77 ± 1360.26	a 0.51 ± 860.08	b 1.03 ± 470.50	b 0.34 ± 172.49	T3
c 2.08 ± 2065.10	ab 1.27 ± 1398.07	a 0.66 ± 869.95	a 0.81 ± 478.50	a 0.99 ± 177.05	T4
b 3.85 ± 2127.29	b 2.29 ± 1393.10	ab 2.58 ± 841.34	b 1.08 ± 467.79	d 0.82 ± 167.79	T5
a 0.74 ± 2159.63	a 2.66 ± 1402.52	a 1.33 ± 866.18	c 0.74 ± 464.37	c 0.33 ± 169.94	T6
**	**	*	**	**	Significance

(P<0.01) ** ،(P<0.05) *

T1 without negative control injection. T2 injection with saline solution Nacl positive control. T3 injection with nano-silver at a concentration of (12 ppm). T4 injection with nano-silver at a concentration of (14 ppm). T5 injection with Nano-silver at a concentration of (16 ppm). T6 injection with nano-silver at a concentration of (18 ppm).

Feed consumption rate

Table (4) shows the effect of various treatments on the feed consumption rate for the different weeks (1-5) of the bird's age. In the first week there was significantly higher (P <0.01) to treatment T1,T3,T4,T5,T6 on T2.in second week was note significantly higher (P <0.01) in T4 treatment compared others and significant T2,T5,T6 on T1,T3 while superior T3 on T1.the table showed no significant difference between the different treatments In the experimental third week. In the fourth week there was significantly higher (P <0.01) in treatment T1 compared with others and significant T2, T5, T6 on T3, and T4 while superiority treatment T4 on T3. In the fifth week the statistical analysis showed significantly higher (P <0.01) in treatment birds T6 on birds treatment T1, T2, T3, T4, and T5 while significant T2, T3, T5 on T3, T4 and significant T4 on T3.in Total feed consumption the treatments T1, T5, T6 its came highest significant (P <0.01) compared with T2, T3, T4, and superior T2, T4 on T3.

Food conversion efficiency

Table (5) shows the effect of injection of hatching eggs with nano-silver in the efficiency of food conversion for the different weeks (1-5) of the bird's age. The first week showed a low significant (P <0.01) for treatment T4 followed by treatment T3 compared to the rest of the treatments. T1, T2, and T6 were not significant. In the second week there was a low significant (P <0.05) in the conversion efficiency of T3,T5 treated birds, followed by T1 on the rest of the birds and low significant in T2,T4 compared with T6.in third week were note low significant (P <0.01) T1, T6 compared others treatment and low significant in T2, T3,T4 compared T5 with no significant between T2, T3,T4.in forth week was low significant (P <0.01) in birds treatment T2,T4 compared with others and low significant in T5,T6 compared with T1,T3while in fifth week we note a low significant (P <0.01) in all treatment injection with nano-silver T3, T4, T5, T6 compared with negative and positive control treatment T1, T2 in the average all treatment injection its came low significant (P <0.05) compared with T1.

Table 3. Effect of injection of hatching eggs in nano-silver in the rate of increase of weight of different weeks (gm).

Mean ± standard error (gm)						Treatment
Total weight increase	fifth week	fourth week	third week	second week	First week	
2.39 ± 1902.78 f	f 1.72 ± 557.50	d 1.46 ± 534.00	ab 1.25 ± 400.02	c 2.07 ± 285.64	c 0.55 ± 125.62	T1
d 4.49 ± 1972.60	2.871 ± 627.65 e	a 0.68 ± 561.22	e 1.06 ± 368.73	bc 1.71 ± 292.31	d 0.37 ± 122.69	T2
e 3.66 ± 1958.89	d 1.10 ± 642.01	f 1.32 ± 500.18	c 0.68 ± 389.58	ab 0.91 ± 298.01	ab 0.86 ± 129.11	T3
c 1.39 ± 2022.10	c 0.71 ± 667.03	e 1.02 ± 528.12	b 1.41 ± 391.45	a 1.12 ± 301.45	a 0.67 ± 134.05	T4
b 1.80 ± 2083.85	b 1.02 ± 734.19	b 0.83 ± 551.76	d 1.20 ± 373.55	a 1.74 ± 300.00	d 0.71 ± 124.35	T5
a 2.01 ± 2116.71	a 0.58 ± 757.11	c 0.62 ± 536.34	a 1.64 ± 401.81	b 0.72 ± 294.43	bc 0.36 ± 127.02	T6
**	**	**	**	**	**	Significance

The averages with different letters within the same column vary significantly between them. ** (P <0.01). T1 without negative control injection. T2 injection with saline solution Nacl positive control. T3 injection with nano-silver at a concentration of (12 ppm). T4 injection with nano-silver at a concentration of (14 ppm). T5 injection with Nano silver at a concentration of (16 ppm). T6 injection with nano-silver at a concentration of (18 ppm).

Percentage of losses and biological ratio

Table (6) shows the effect of different coefficients in the total percentage of losses and the biological ratio. No significant differences were observed between the different treatments in the percentage of loss and the biological ratio. The superiority of live body weight, weight gain, and improved dietary conversion efficiency may be due to the nano-silver antibacterial properties [16], which improved the intestines environment and increased nutrient uptake [9] , where they confirmed that nano-silver improved the growth of birds as a strong antibacterial antibody is also an antioxidant ,which is reflected in the weight of birds and the efficiency of food conversion, while the superiority in feed consumption may be due to the high increase in body weight of the treatment nano-silver injection T6 that weight increase requires a higher amount of feed to meet the needs The energy of the bird at the same time treatment control taken amount of feed with poor food conversion efficiency and the nano-silver in the injection treatments resulted in an improvement a study we did found that nano-silver improved the length and width of the intestine and

increased surface area in the intestine, which improved the absorption process and thus increased the efficiency of feed consumption compared to the treatment of negative control T1 and not compatible with [2-10-14] , who reported that nano-silver did not affect growth, but improved the performance of the thrombocytopenia and its immune system, which indicates that the nanosilver had a selective effect on the microflora of the digestive tract in the chickens [1] noted that nano-silver reduced dietary conversion efficiency but significantly increased (P <0.05) weight of small intestine and abdominal fat in broilers compared with control group. This effect may be due to the nano-silver effect on intestinal bacteria [23-6] and antibiotic-resistant strains [22-14]. Gram-negative bacteria include species such as Acinetobacter, Escherichia , Pseudomonas, Salmonella, Vibrio This is based on studies that showed that silver nanoparticles The cell wall destroys the gram-negative bacteria [12-16].

Table 4: Effect of injecting hatching of eggs in nano-silver in the feed rate (g / birds) for different weeks

Mean ± standard error (gm)						treatment
Total consumer feed	**fifth week**	**fourth week**	**third week**	**second week**	**First week**	
ab 5.04 ± 2946.48	b 2.92 ± 1091.64	a 2.59 ± 830.98	a 1.31 ± 544.85	d 1.06 ± 346.01	a 0.99 ± 133.01	T1
28.92 ± 2910.55 bc	b 3.24 ± 1086.04	b 2.95 ± 816.19	a 23.97 ± 522.89	bc 2.01 ± 355.85	b 0.50 ± 129.56	T2
d 2.71 ± 2814.11	d 2.67 ± 1016.85	d 3.04 ± 764.07	a 1.63 ± 546.76	c 1.09 ± 353.00	a 0.39 ± 133.43	T3
c 7.56 ± 2880.89	c 2.47 ± 1043.10	c 3.95 ± 782.09	a 1.44 ± 554.25	a 1.11 ± 368.67	a 0.66 ± 134.77	T4
ab 3.22 ± 2927.73	b 1.43 ± 1085.20	b 1.45 ± 815.18	a 1.37 ± 540.01	bc 1.25 ± 354.84	a 0.96 ± 132.49	T5
a 6.55 ± 2954.97	a 2.95 ± 1103.99	b 3.49 ± 807.83	a 1.34 ± 550.13	b 2.63 ± 359.62	a 0.83 ± 133.39	T6
**	**	**	NS	**	**	significance

** P<0.01 ،(NS: Not significant)

T1 without negative control injection. T2 injection with saline solution Nacl positive control.
T3 injection with nano-silver at a concentration of (12 ppm). T4 injection with nano-silver at a concentration of (14 ppm). T5 injection with Nano silver at a concentration of (16 ppm). T6 injection with nano-silver at a concentration of (18 ppm).

Injection treatments may be due to the inhibition of the growth of pathogenic microorganisms and the reduction of their activity by nano-silver, thus contributing effectively to the reduction of pathological injury [6-11-13-23], thus reducing losses, as confirmed [10].

Table 5 : Effect of injection of nano-silver hatching eggs in food conversion efficiency for different weeks (kg feed / kg meat / birds)

Mean ± standard error (kg feed / kg meat / bird)						treatment
Average	**fifth week**	**fourth week**	**third week**	**second week**	**First week**	
a 0.05 ± 1.429	a 0.25 ± 1.958	a 0.006 ± 1.556	c 0.009 ± 1.362	bc 0.008 ± 1.211	b 0.009 ± 1.058	T1
b 0.002 ± 1.374	b 0.007± 1.730	e 0.004 ± 1.454	b 0.007 ± 1.418	bc 0.006 ± 1.217	b 0.006 ± 1.055	T2
b 0.003 ± 1.346	c 0.008 ± 1.583	ab 0.004 ± 1.527	b 0.006 ± 1.403	c 0.004 ± 1.184	0.004 ± 1.033 bc	T3
b 0.002 ± 1.337	c 0.008 ± 1.563	cd 0.006 ± 1.480	b 0.004 ± 1.415	ab 0.006 ± 1.222	c 0.004 ± 1.005	T4
b 0.002 ± 1.329	c 0.008 ± 1.478	d 0.008 ± 1.477	a 0.006 ± 1.445	c 0.006 ± 1.182	a 0.007 ± 1.065	T5
b 0.003 ± 1.320	c 0.008 ± 1.458	bc 0.010 ± 1.506	c 0.004 ± 1.369	a 0.009 ± 1.221	b 0.004 ± 1.050	T6
*	**	**	**	*	**	significance

The averages with different letters within the same column vary significantly between) *P<0.05 ** ، (.(P<0.01) them. T1 without negative control injection. T2 injection with saline solution Nacl positive control. T3 injection with nano-silver at a concentration of (12 ppm). T4 injection with nano-silver at a concentration of (14 ppm). T5 injection with Nano silver at a concentration of (16 ppm). T6 injection with nano-silver at a concentration of (18 ppm).

Table 6. Effect of injection of hatching eggs in nano-silver in the Total Percentage of Depreciation (%) and biological percentage %

Average ± standard error		Treatment
(%) biological percentage	(%) Total Percentage of Depreciation	
0.05 ± 99.00	0.05 ± 1.00	T1
0.25 ± 99.50	0.25 ± 0.50	T2
0.25 ± 99.50	0.25 ± 0.50	T3
0.00 ± 100.00	0.00 ± 0.00	T4
0.00 ± 100.00	0.00 ± 0.00	T5
0.00 ± 100.00	0.00 ± 0.00	T6
NS	NS	Significance

‹(NS: Not significant). T1 without negative control injection. T2 injection with saline solution Nacl positive control. T3 injection with nano-silver at a concentration of (12 ppm). T4 injection with nano-silver at a concentration of (14 ppm). T5 injection with Nano silver at a concentration of (16 ppm). T6 injection with nano-silver at a concentration of (18 ppm).

REFERENCES

[1] A h m a d i F., R a h i m i F. (2011). The effect of different levels of nano silver on performance andretention of silver in edible tissues of broilers. World Appl. Sci., 12:1–4

[2] Andi, M.A.; Mohsen, 2011 H.; Farhad, A. Effects of feed type with/without nano-silver on cumulative performance: Relative organ weight and some blood parameters of broilers. *Glob. Vet.* 2011, *7*, 605–609.

[3] A h m a d i F., K h a h M.M., J a v i d S., Z a r n e s h a n A., A k r a d i L.,S a l e h i f a r P. (2013). Theeffect of dietary silver nano particles on performance, immune organs, and lipid serum of broiler chickens during starter period. Inter. J. Biosci., 3: 95–100.

[4] AL-Fayyad, Hamdi Abdul Aziz and Naji, Saad Abdul Hussein. Poultry products technology 1989. Higher Education Edition - University of Baghdad.

[5] AL-Zubaidi, Suhaib Saeed Alwan .. 1986 Poultry management. Basra University Press

[6] Burrell RE, JP Heggers, GJ Davis and JB Wright, 1999. Efficacy of silvercoated dressings as bacterial barriers in a rodent burn sepsis model. Wounds, 11: 64-71

[7] Duncan, D.B. 1955. Multiple Rang and Multiple F-test. Biometrics. 11: 4-42.

[8] Rai M, Yadav A, Gade A. 2009. Silver nanoparticles as a new generation of antimicrobials.Biotechnol Adv. 27:76–83.

[9] Güllüce M, Sökmen M, Daferera D, Agar G, Özkan H, Kartal N, Polissiou M, Sökmen A & Sahin F. 2003. In vitro antibacterial, 172 Ovo injection of nano-silver, thyme and savory antifungal, and antioxidant activities of the essential oil and methanol extracts of herbal parts and callus cultures of Satureja hortensis L. Journal of Agricultural and Food Chemistry, 51: 3958 -3965.

[10] Katarzyna Ognik1♦, Iwona Sembratowicz1, Ewelina Cholewińska1, Łukasz Wlazło2,Bożena Nowakowicz-Dębek2, Radosław Szlązak3, Krzysztof Tutaj4 The effect of chemically-synthesized silver nanoparticles on performance and the histology and microbiological profile of the jejunum in chickens* *Ann. Anim. Sci., Vol. 16, No. 2 (2016) 439–450 DOI: 10.1515/aoas-2015-0067*

[11] Lansdown, A.B.G. A review of the use of silver in wound care: Facts and fallacies. *Br. J. Nurs.* 2004, *13*, 6–19.

[12] Morones JR, JL Elechiguerra, A Camacho, K Holt, JB Kouri, JT Ramırez and MJ Yacaman, 2005. The bactericidal effect of silver nanoparticles. Nanotechnology, 16: 2346-2353 .

[13] Ovington, L.G. The truth about silver. *Ostomy Wound Mag.*2004, *50*, 1–10.

[14] Percival SL, PG Bowler and J Dolman, 2007. Antimicrobial activity of silver-containing dressings on wound microorganisms using an in vitro biofilm model. Int Wound J, 4: 186-191

[15] Sawosz, F.; Gao, Y. 2012; Ali, A.; *et al*. Effect of silver nanoparticles on growth performance; metabolism and microbial profile of broiler chickens. *Arch. Anim. Nutr.* 2012, *66*, 416–429.

[16] Sondi I, B Salopek-Sondi, 2004. Silver nanoparticles as antimicrobial agent: A case study on E. coli as a model for Gram negative bacteria. J Colloid Interface Sci, 275: 177-182.

[17] SAS. 2012. Statistical Analysis System, User's Guide. Statistical. Version 9.1th ed. SAS. Inst. Inc. Cary. N.C. USA.

[18] Sanvicens N, Marco MP. 2008. Multifunctional nanoparticles – properties and prospects for their use in human medicine. Trends Biotechnol. 26:425–433.

[19] St-Pierre, N. R., B. Cobanov, and G. Schnltkey. 2003. Economic Losses from heat stress by us livestock industries. J. Dairy . Sci. 86 :(E. Suppl.) : E52-E77.

[20] Subrat K. Bhanja 1, Anna Hotowy 2, Manish Mehra 1, Ewa Sawosz 3, Lane Pineda 2,Krishna Prasad Vadalasetty 2, Natalia Kurantowicz 3 and André Chwalibog2015 ,**In Ovo* Administration of Silver Nanoparticles and/or AminoAcids Influence Metabolism and Immune Gene Expression inChicken Embryos.+45-3533-3044; Fax: +45-3533-3020.Academic Editor: Bing Yan. Received: 15 March 2015 / Accepted: 21 April 2015 / Published: 27 April 2015

[21] US.EPA.2010.United States Environmental Protection Agency. www . epa.gov

[22] Wright JB, K Lam, AG Buret, ME Olson and RE Burrell, 2002. Early healing events in a porcine model of contaminated wounds:Effects of nanocrystalline silver on matrix metallo proteinases , cell apoptosis, and healing. Wound Repair Regen, 10:141-151

[23] Yin HQ, R Langford and RE Burrell, 1999. Comparative evaluation of the antimicrobial activity of ACTICOAT antimicrobial barrier dressing. J Burn Care Rehabil, 20:195-200.