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EFFECT OF SOME MEDICINAL PLANTS ON GROWTH PARAMETERS AND HEALTH STATUS OF NILE TILAPIA (*Oreochromis niloticus*).

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ABSTRACT

This experiment was carried out to determine the effect of adding 0, 0.25, 0.50, 0.75, and 1% of dry thyme *(Thymus vulgaris*) and sage *(Salvia officinalis*) leaves to the ration of Nile tilapia (*Oreochromis niloticus*) on growth performance, feed and nutrient utilization, whole body composition, hematogram, protein profile and the activity of serum enzymes. Fingerlings of 15g average weight were stocked in 27 glass aquaria (105x32x40cm) at a rate of 15 fish in each. Fish in each aquarium were handly fed on the experimental pelleted feeds twice daily, six days a week at a rate of 3% of body weight daily for a period of 105 days. The results showed that, nutrient utilization, growth performance, hematogram, protein profile and activity of serum enzymes were improved with adding the thyme at level of 0.25 and 0.5% and sage at 0.5% and 0.75%. Also, fish fed rations containing 0.25,0.5, 0.75. and1% thyme or 0.75, and 1% sage had significantly ($P<0.05$) lower body fat, ash and energy contents but significantly ($P<0.05$) higher dry matter and body protein content.There were no significant mortalities among fish groups during the experimental period. Therefore, the levels of 0.25 or 0.5% thyme and 0.25, 0.5 or 0.75% sage are recommended as growth promoters for Nile tilapia due to their favorable effects on growth performance and health status.

Keywords: Nile tilapia-Medicinal feed additives-Growth performance.

INTRODUCTION

Herbs and herbal extracts contain active phytochemicals that promote health and protect against chronic diseases (Craig, 1999). The average annual planted area of medicinal herbs in Egypt is 44.804feddan (Ministry of Agriculture Report, 2003). Recently, it has been found that, herds such as thyme or sage have some medical properties and pharmacological activities that could help in improving both growth and health conditions. There is a tendency to use medicinal plants as natural feed additives to avoid the residual cumulative effect of antibiotic growth promoters and chemicals in fish (Mohamed., 1999, Shalaby *et al.*, 2003; Lewis and Ausubel; 2006 and Mousavi *et al.*, 2009).

Thyme *(Thymus vulgaris)* and sage *(Salvia officinalis)* have highly aromatic odor with a pungent and slightly bitter taste. They cause a significant improvement in body weight, feed conversion, and mortality rate (Tollba, 2003 and Gibbons, 2005). Thyme leaves contain from 1.10 to 1.40% essential oils. The main active constituents of the essential oil are thymol (40 to 60%) and carvacrol (1 to 5%) (Radwan, 2003 and Gibbons, 2005). Moreover, thyme are rich in essential oils as well as their monoterpene components; α-pinene and β-pinene (Muhlbauer *et al.,* 2003). Essential and active constituents are yellowish

volatile oil (1 to 2.5%) containing thymol, carvacrol, cymene, pinene, borneol, linalool, bornyl acetate, tannin, bitter principle, saponin, sugars, flavone, caffeic acid and ursolic acid which is the isomer of oleanolic acid. Hassanein (1982) found that the oil contents of Egyptian thyme plant were 2.07% (on dry weight basis) and the essential oil extracted by GLC contained 25.65% thymol (phenols), 2.6% limonene, 19.2% p-cymene and 7.2% cyclic terpenes. Lima *et al.* (2004) and Menaker *et al.* (2004) isolated essential oil (E.O.) of sage (*Salva officinalis*) from air-dried vegetative aerial parts of the plants by hydro distillation and extracted a total yield of 12.07 mg of E.O./g plant (dry mass) and more than 50 compounds were identified. The maior compounds were cis-thujone (17.4%), alpha-humulene (13.3%), 1,8-cineole (12.7%), E-caryophllene (8.5%) and bomeal 8.3%. The present study was carried out to evaluate the effect of feeding graded levels of some medicinal plants (mainly thyme and sage at, 0.25, 0.5, 0.75, and 1%) on the growth performance, feed utilization, body composition, hematogram, protein profile and activity of serum enzymes for Nile tilapia (*O. niloticus*).

MATERIALS AND METHODS

The present work was applied in the Aquaculture Research Unit, Sakha, Kafr El-Sheikh governorate in year 2009 in order to evaluate some medicinal plants (thyme and sage) at dietary levels of 0.25, 0.5, 0.75 and 1% for Nile tilapia (*Oreochromis niloticus*) and their effects on growth performance, feed utilization, body composition, under the Egyptian conditions.

1- Fish culture system:

Twenty-seven aquaria were used in the present study, each measuring (105x32x40cm) with total volume of 134 liters. A total of 405 fingerlings (obtained from a private fish farm in Kafr El-Sheikh, Egypt) fish of Nile tilapia (*Oreochromis niloticus*) with 15g average body weight were used at a stoking density of 15 fish/ aquarium. They were maintained in the aquaria for one month before the beginning of the experiment for acclimation purpose. Fish were fed during the acclimation period on an artificial basal ration 26.54% CP at a rate of 3% of the body weight at 2 meals daily. Water was partially changed once every day, using dechlorinated fresh water. Aeration was provided using air blower. Each treatment was applied in 3 replicates

2- Experimental rations:

Fingerlings were fed the experimental rations containing 26.54% CP and 443.544 GE kcal/100g at a feeding rate of 3% of fish biomass in each aquarium (six days per week). Fish were fed twice daily at (8 a.m and 2 p.m) with feed amounts adjusted biweekly according to the weight development. A basal ration was formulated from the commercial local ingredients (fish meal, soybean meal, yellow corn, wheat bran, vitamin-mineral mixture and oil). The dry ingredients were grounded through a feed grinder to small particles (0.1mm). The ingredients were weighed and mixed by a dough mixer for 20 minutes to homogenise the ingredients. The estimated amount of oil was gradually added (few drops gradually) and the mixing operation was continued for 20 minutes. After a homogenous mixture was obtained, 40ml water/ 100g ration were slowly added to the mixture according to Shimeino *et*

al. (1993). The rations were cooked on a water evaporator for 20 minutes. The different doses of thyme and sage(0.25, 0.5, 0.75, and 1%) were added to the ingredients prior to pelletization. The chemical analysis of thyme and sage levels and their oils are given inTables 2 and 3, respectively

The rations were pelleted through a fodder machine and the pellets were dried under room temperature for 24 h before use. The required amount of the ration was prepared every two weeks and stored in a refrigerator at 4Ċ. Chemical analysis of feed ingredients used in the experimental rations is presented in Table (1). These rations were designated as ration 1 to 9 They were isonitrogenous (26.54%) and isocaloric (443.544 GE kcal/100g). Composition of the mixed rations is presented in Table (4).

Table (1): Proximate analysis of the dietary ingredients (% on DM basis).

Ingredients	DM	CP	EE	CF	Ash	NFE%*	GE Kcal/100kg**
Fish meal	92.1	72.0	8.7	-	12.6	6.70	512.798
Soybean meal	92.4	44.0	1.2	5.8	6.5	42.5	431.978
Yellow corn	89.2	8.8	3.8	2.6	1.8	83.0	425.452
Wheat bran	88.2	11.9	3.0	11.0	5.0	69.1	-378.270

***NFE= 100-(CP+EE+CF+Ash).**

**** Gross energy was calculated by multiplication the factors 4.1, 5.6 and 9.44 kcal/g GE/g DM of carbohydrate, protein and fat, respectively (Jobling,1983).**

Table (2):Chemical composition of thyme and sage leaves:

Item	DM	CP	EE	CF	ASH	NFE	Auther
Thyme	88.48	20.19	4.00	19.40	30.00	26.41	Schleichr, *et al*, 1998
	27.47	13.25	3.63	16.56	35.00	31.56	Abaza, 2001
	89.59	6.19	4.98	16.36	30.50	41.97	Radwan, 2003
Sage	88.56	11.00	3.40	19.40	11.80	54.40	Niki 1992
	88.57	11.00	3.80	18.80	11.50	54.90	Ibrahim *et al.*, 2002
	89.00	9.24	4.00	19.50	12.11	55.15	Eisa, 2004

3-Growth parameters:

Average total gain (ATG), average daily gain (ADG), specific growth rate (SGR), feed conversion ratio (FCR), protein efficiency ratio (PER), protein productive value (PPV %) and survival rate (SR %) were calculated according to the following equations:

a- ATG (g/fish) = [Average final weight (g) – Average initial weight (g)] as reported by (Annet., 1985). **b-** ADG (g/fish/day) = [ATG (g)/experimental period (d) **c-** SGR (%/day) = [Ln final body weight–Ln initial body weight] x 100/experimental period (d) according to Pouomonge and Mbongland (1993). **d-** FCR= Feed intake, dry weight (g)/live weight gain as reported by De Selva and Anderson (1995). **e-** PER = Live weight gain (g)/ protein intake (g) as reported by De Selva and Anderson (1995). **f-** PPV (%) = 100[final fish body protein (g) – initial fish body protein (g)/crude protein intake (g). **g-** SR = 100[total No. of fish at the end of the experiment /total No. of fish at the start of the experiment.

4 – Proximate analysis:

Dry matter, crude protein, ether extract, crude fiber and ash contents of the feed ingredients, experimental rations and whole body of fish at the beginning and at the end of the experiment were performed according to A.O.A.C. (1990).

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5- Clinico pathological examination:

Blood samples were collected from the caudal vein, part of blood was left to clot and then centrifuged at 3000 r.p.m. to obtain the serum for biochemical studies according to Goldenfarb, *et al*. (1971). The other part was heparinized for hematological investigations using the methods of Blaxhall and Daisley, (1973)..

6- Statistical analysis:

The obtained numerical data were statistically analyzed using SPSS (1997) for one-way analysis of variance. When F- test was significant, treatment means were compared according to Duncan *(*1955).

RESULTS AND DISCUSSION

1- Water quality parameters:

The most important physico-chemical parameters of tap water used in the experiment are shown in Table (5). Data in this Table indicate that the values obtained lie in the acceptable ranges required for normal growth of tilapia (AbdEl-Hakim *et al.*, 2002 and Abdelhamid *et al.,* 2006).

Table (5): Ranges of physico-chemical parameters measured in fish-rearing-water throughout the experimental period.

Temperature (ċ)	pH value	DO2 Ppm	Alkalinity mg/l	Hardness mg/l	PO4 mg/l	NO2 mg/l	NO3 mg/l
27-28	7-8.5	5-6	140-160	300-350	0.2-.03	0.10-0.12	1-3

2- Growth performance:

Results present in Table (6) show that values of TWG, ADG, and SGR did not differ significantly (P>0.05) among rations 1 (0.25% thyme), 2 (0.5% thyme), 5 (0.25% sage), 6 (0.5% sage), and ration 7 (0.75% sage), and SR did not differ significantly (P>0.05) among treatments. But the results clearly show that the rations containing (0.25% and 0.5% thyme) and (0.25%, 0.5% and 0.75% sage) were slightly better in average weight gain, average daily gain and specific growth rate than the control ration and other treatments (0.75%, 1% thyme and 1% sage). The results herein are in agreement with the findings of Abd El-Latif *et al.* (2002) who considered that thyme and sage as nontraditional feed additives, or growth promoters had a significant improving effect on body weight, weight gain, mortality rate and feed conversion.

3- Feed and protein utilization:

Data of feed and protein utilization expressed as feed intake (FI), feed conversion ratio (FCR), protein efficiency ratio (PER) and protein productive value (PPV) are given in Table (7).

The data indicated that FCR, PER, and PPV did not differ significantly among rations T1, T2 (*thyme*), T5, T6, and T7 *(sage*), but FCR, PER, and PPV showed significant (P≤0.05) increases for the rations T1, T2, T5,T6, and T7 as compared with the control and the other treatments. The variations in the previously recorded results may be attributed to that the doses of both thyme and sage which play an important role in either being of a healthy additive or not. This interpretation are in agreement with those reported by Diab *et al.* (2006).

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Table (7): Feed and nutrient utilization of Nile tilapia fed on the experimental rations containing different levels of *thyme* and *sage* (Mean±SE).

Treatment	Feed Intake	FCR	PER	PPV
Thyme				
*(control)	46.50±1.50	1.51±0.44 a	2.55±0.26 b	28.94±0.75 b
1- (0.25%)	47.50±1.00	1.10±0.12 b	3.49±0.12 a	32.36±0.42 a
2- (0.5 %)	45.70±2.50	1.35±1.00 b	2.84±0.14 a	37.03±0.40 a
3- (0.75%)	47.00±1.00	1.58±0.10 a	2.44±0.18 b	27.82±0.48 b
4- (1%)	44.45±2.00	1.83±0.09 a	2.41±0.16 b	22.00±0.60 b
Sage				
5- (0.25%)	48.50±1.00	1.05±0.50 b	3.65±0.28 a	35.68±0.55 a
6- (0.5%)	46.70±1.60	1.17±0.04 b	3.29±0.25 a	35.42±0.45 a
7- (0.75%)	46.00±1.02	1.29±0.05 b	2.98±0.12 a	29.00±0.47 a
8- (1%)	43.45±2.00	1.56±0.08 a	2.47±0.10 b	27.20±0.56 b

a,b means in the same column bearing the same letter do not differ significantly at 0.05 level.

4- Body composition:

Values of dry matter (DM), crude protein (CP), ether extract (EE) and ash of the fish body are summarized in Table (8). The results of carcass composition of Nile tilapia showed no significance (P>0.05) in dry matter, crude protein and ash among fish treatments. Meanwhile, the percentage of ether extract differed significantly among fish groups which may be attributed to the presence of phenolic compounds that act as natural antioxidants. Such remarks are in compatibility with the results of Ho (1992) and Amiot *et al.* (1997).

Table (8): Means ± standard error of proximate analysis (% on the dry matter basis) of experimental fish fed on graded levels of thyme and sage.

Treatments	DM	CP	EE	Ash
Thyme				
*(control)	26.38±0.18 a	59.00±0.83a	2.38±0.95b	18.62±0.75a
1- (0.25%)	22.89±1.16 a	59.50±0.30 a	24.05±0.27a	16.45±0.07a
2- (0.5 %)	25.61±0.30 a	59.63±1.20 a	23.25±0.02ab	17.12±1.68a
3- (0.75%)	24.23±0.11 a	58.97±0.25 a	21.83±0.02b	19.20±0.23a
4- (1%)	26.03±0.22 a	58.02±0.88 a	21.99±0.19b	19.99±0.01a
Sage				
5- (0.25%)	25.00±0.50a	59.00±1.83 a	24.00±0.15a	17.00±0.18a
6- (0.5%)	24.89±0.15 a	58.80±0.30 a	24.65±0.18a	16.55±0.10a
7- (0.75%)	25.00±0.36 a	59.60±1.20 a	23.30±0.02ab	17.10±0.68a
8- (1%)	28.23±0.10 a	59.70±0.25 a	22.20±0.02b	18.10±0.12a

A and b means in the same column bearing the same letter do not differ significantly at 0.05 level.

7- Hematogram, protein profile and enzymes activity:

The results of hematogram and protein profile showed a significant increase in red blood cells (RBC), white blood cells (WBC), haemoglobin content (HB), packed cell volume (PCV), total protein, albumin and globulin. While there was significant decrease in serum enzymes activity (ALT and AST) on feeding levels of (0, 0.25, 0.5, and 0.75%) compared with 1% *of either thyme* or *sage* which gave elevated levels of AST and ALT. Best treatments were obvious in fish fed rations containing *thyme* or *sage* at concentrations of 0.25, 0.5, and 0.75% (Table 9).

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Therefore, the improvement of the previously mentioned physiological parameters means more ability for disease resistance. These results and remarks are in agreement with those reported by Shalaby *et al.* (2003), Gibbons (2005), and Mousavi, *et al.* (2009).

CONCLUSION

From the feed utilization data, and evaluation of health status the ration supplemented with 0.25 and 0.5% thyme and 0.25, 0.5% and 0.75% sage were the best treatments.

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أثر إضافة مسحوق الزعتر والمريمية في علائق اسماك البلطي النيلي على أداء النمو و الحالة الصحية.

محمود عثمان الجندي، هشام محمد الكومى، محمد تاج الدين شهاب الدين، عادل عزت طولان و محمود فؤاد إسماعيل سالم.

المعمل المركزى لبحوث الثروة السمكية - وحدة بحوث الثروة السمكية بسخا, مصر

تم إجراء هذا البحث بمعمل وحدة بحوث الثروة السمكية بسخا وذلك بغرض دراسة بعض الإضافات الغذائية (الزعتر و المريمية) وتم إضافتها إلى علائق اسماك التجربة بتركيزات (0.25% , 0.5%, 0.75% و 1%) وهى نباتات طبية لدراستها كمنشطات للنمو في علائق أسماك البلطي النيلي ,واستغرقت مدة هذه التجربة 105 يوما تم فيها تغذية الأسماك بمعدل3% على مرتين يوميا في الثامنة صباحا والثانية مساء. ومن أهم القياسات التي تم أخذها هي معدلات النمو وتركيب الجسم بالإضافة لبعض قياسات الدم الهامة. ومن أهم النتائج التي تم التوصل إليها من خلال هذه الدراسة أن هذه الإضافات (الزعتر والمريمية) بتركيزات (0.25% و 0.5% مسحوق زعتر) و(0.25% و 0.5% و 0.75% مسحوق مريمية) حققت أحسن معدلات للنمو وأحسن قياسات لتركيب الجسم و مكونات الدم. لذلك من خلال هذا البحث يمكن أن ننصح بإضافة هذه النباتات الطبية كمنشطات للنمو وتحسين للصحة العامة في علائق أسماك البلطي النيلي.

قام بتحكيم البحث

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Table (3): Essential oil and active constituents in thyme and sage:

Item	Phenol acid. %	carvacrol %	Opignin Mg/kg	Luteolin Mg/kg	P-cymene %	δ- Terpinene %	Limonene %	Auther
Thyme	51.00	2.95	50	51	21.00	13.00	2.8	Doman,*et al.* and Deans, 2000
	39.37	1.47	52	51	20.58	12.79	2.00	Justesen and knuthsen 2001
	40.60	1-5	50	50	21.50	12.50	2.70	Radwan, 2003
	57.90	2.00	50	50	16.67	13.00	2.30	Mohamed, 1999
Sage	**β-Pinene %**	**Evcalyptol %**	**α- Thujone %**	**B- Thujone %**	**Comphor %**	**Bornyl acetate %**	**1,8-cineole%**	
	0.23-2.02	4.98-13.40	35.90-45.80	4.35-9.6	15.50-21.15	0.82-2.72	12.8-13.11	Piccagliad, 1993
	1.11-2.00	3.50-12.00	36.40-50.51	8.4-8.8	15.40-16.20	0.55-1.20	12.1-14.00	Vora *et al*, 1993
	2.20-3.00	4.00-13.00	43.30-45.50	17.4-18.00	16.50-18.26	0.45-2.14	13.10-13.40	Menker *et al.*, 2004
	0.50-2.44	4.21-12.10	42.30-46.22	5.00-10.50	15.50-17.44	0.50-1.46	12.50-13.20	Farag *et al.*, 1989

Table (4): Ingredients and composition of the experimental rations.

Ingredient	Diet1	Diet2	Diet3	Diet4	Diet5	Dirt6	Diet7	Diet8	Diet9
Fish meal	10	10	10	10	10	10	10	10	10
Soybean meal	38	38	38	38	38	38	38	38	38
Yellow corn	32	32	32	32	32	32	32	32	32
Wheat brean	15	15	15	15	15	15	15	15	15
Sunflower oil	4	4	4	4	4	4	4	4	4
Vit.&min.[1]	1	1	1	1	1	1	1	1	1
Thyme	-	0.25	0.5	0.75	1	-	-	-	-
Sage	-	-	-	-	-	0.25	0.5	0.75	1
Chemical analysis (%):									
Dry matter	89	90	88	87	90	86	89	87	85
Crude protein	26.54	26.84	26.55	26.65	26.75	26.20	26.80	26.50	26.00
Ether extract	9.5	8.5	9.00	9.50	9.70	9.55	9.45	9.56	9.55
CF	5.5	6.5	5.75	5.65	5.50	5.51	5.56	5.58	5.50
Ash	8.4	7.45	8.45	8.68	8.55	8.50	8.30	8.50	8.00
NFE	50.06	50.71	50.25	49.52	49.50	50.24	49.89	49.86	50.95
Calculated values:									
GE kcal/100 g [2]	443.544	436.83	439.39	441.95	444.31	442.85	443.82	443.06	444.64
P/E ratio (mg protein/kcal)3	59.83	61.44	60.42	60.30	60.20	59.16	60.38	59.81	58.47

(1)Vitamin and mineral mixture (product of HEPOMIX) each 2.5 kg contain : 12.000.000 IU Vit.A; 2.000.000 IU Vit . D3 ; 10 g Vit. E ; 2g Vit. K3 ; 1g Vit. B1 5g Vit. B2;1.5 g Vit. B 6 ; 10g Vit.B12; 30 g Nicotinic acid ; 10 g Pantothenic acid ; 1g Folic acid; 50g Biotien; 250g Choline chlorid 50% ; 30g Iron; 10g copper; 50g Zinc; 60g Manganese; 1g Iodine; 0.1g Selenium and Cobalt 0.1g.

(2) GE (gross energy) calculated using the values 4.1 , 5.6 and 9.44 Kcal GE/g DM of carbohydrate , protein and fat, respectively (Jobling, 1983).

(3) P/E (protein to energy ratio) = mg crude protein / Kgcal GE.

Table (6): Growth performance parameters ($\bar{x}$±SE) of Nile tilapia fed the experimental rations containing different levels of thyme and sage.

Treatment	Initial Weight(g)	Final Weight(g)	TWG (g/fish)	ADG (g/fish/day)	SGR (%/day)	SR (%)
Thyme						
***(control)**	15.50±0.50	46.5±3.20 c	30.80± 1.20 c	0.29±0.01c	1.14±0.19c	100±0.00
1- (0.25%)	15.50±0.50	58.6±0.20 a	43.10± 0.13 a	0.41±0.03 a	1.27±0.15a	100±0.00
2- (0.5 %)	15.50±0.50	49.3±0.3 ab	33.80 ±0.43ab	0.32±0.04ab	1.20±0.28ab	100±0.00
3- (0.75%)	15.50±0.50	45.3±0.02 c	29.80± 0.46 c	0.28±0.04 c	1.02±0.01c	100±0.00
4- (1%)	15.50±0.50	39.8±.40 d	24.30±1.00 d	0.23±0.02 d	0.89±0.12d	100±0.00
Sage						
5- (0.25%)	15.50±0.50	61.6±0.20 a	46±1.14a	0.43±0.04a	1.51±0.25a	100±0.00
6- (0.5%)	15.50±0.50	55.50±0.3 ab	40±0.12ab	0.38±0.06ab	1.21±0.22ab	100±0.00
7- (0.75%)	15.50±0.50	51.20±0.02 b	35.7±0.18b	0.34±0.03b	1.13±0.15b	100±0.00
8- (1%)	15.50±0.50	43.40±.40 c	27.9±0.45c	0.26±0.02c	0.98±0.14c	100±0.00

a, b, c d: means in the same column bearing the same letter do not differ significantly at 0.05 level.
*** (Control group), T1= (basal ration + 0.0.25% *thyme*), T2= (basal ration + 0.*5% thyme*), T3=(basal ration + 0.0.75% *thyme*), T4 = (basal ration + 1% *thyme*), Ration5 = (basal ration + 0.0.25% *sage*), Ration6 = (basal ration + 0.5% *sage*), Ration7 = (basal ration + 0.75% *sage*), and Ration8 = (basal ration + 1% *sage*).**

Table (9): Means ± SE of some hematological and biochemical parameters at the end of the 15 weeks experimental feeding of the tilapia fish:

Trs.	RBCs (10^6/mm)	WBCs (10^3/mm)	Hb (g%)	PCV%	Total protein (g/dl)	Albumin g/dl	Globuline g/dl	AST U/L	ALT U/L
Thyme									
*(control)	0.71±0.01b	60.50±0.50c	5.50±0.50a	25.50±0.50ab	3.35±0.55a	1.05±0.001c	2.30±0.05ab	78.50±0.50ba	34.50±0.50a
1- (0.25%)	0.77±0.01a	63.50±0.50ab	5.30±0.10a	27.50±0.50a	3.40±0.05a	1.00±0.001c	2.40±0.001a	70.50±0.50b	21.00±0.00b
2- (0.5 %)	0.76±0.005ab	62.00±0.00bc	5.15±0.50a	25.50±0.50ab	3.20±0.00b	1.20±0.005b	2.00±0.001ab	67.00±1.00a	22.00±0.00b
3- (0.75%)	0.75±0.005ab	61.50±0.50c	5.35±0.05a	27±0.00a	3.45±0.005a	1.30±0.10b	2.15±0.001b	67.50±2.50a	24.50±0.50a
4- (1%)	0.54±0.005c	52.50±0.50d	4.10±0.00	18.50±0.50c	3.05±0.005b	1.10±0.005c	1.95±0.05bc	93.50±1.50	29.00±1.00a
Sage									
5- (0.25%)	0.79±0.005a	64.50±0.00a	5.50±0.01a	32.50±1.15a	3.35±0.005a	1.25±0.001b	2.10±0.001ab	70.50±0.50b	23.00±0.00b
6- (0.5%)	0.75±0.005ab	61.50±0.50c	5.35±0.05a	28.50±0.50a	3.35±0.005a	1.55±0.05a	1.80±0.001c	77.00±0.90a	21.50±0.50b
7- (0.75%)	0.62±0.005b	54.00±1.00d	3.95±0.05b	26..50±0.50ab	3.20±0.00b	1.50±0.001a	1.70±0.001cd	87.00±0.50a	23.50±1.50b
8- (1%)	0.54±0.005c	52.11±0.50d	3.80±0.00b	17.00±1.00c	2.90±0.005b	1.60±0.001a	1.30±0.003d	95.00±1.00a	33.50±1.50a

a, b and c means in the same column bearing the same letter do not differ significantly at 0.05 level.