

Protective Effect of Moringa and Sage on Fertility Impairment Induced by Synthetic Pyrethroids in Male Rats

By

¹Samar, H. Sh. El-Defrawy; ²Hanan ,M. Sobhy; ³Thanaa, A. El-Kholy;
⁴Samah, A. El-Hashash; and ⁵Hanan,A .Sultan

¹Nutrition and food science.Dep-Faculty of Home Economics Al-Azhar university

²BiochemToxicol& Food Deff.Dep - Animal Health Research Institute

^{3,4}Nutrition and food science. Dep – Faculty of Home Economics Al – Azhar university.

⁵ Nutrition Dep- Research Institute of Ophthalmology(RIO)

ABSTRACT

The main objective of this study was to investigate the efficiency of Moringa and sage leaves as protective agents on deltamethrin-induced morphological and functional changes in gonads of male albino rats. Dry Moringa and sage leaves were chemically analyzed in order to identify their nutritive values. For the biological study fifty four adult normal male albino rats (Sprague Dawley strain) of an average body weight 150-180 g were divided into 9 groups of 6 rats each including control (-ve) group, control (+ ve) group 1 (administered deltamethrin orally at 10mg/kg b.wt.), control (+ ve) group 2 (administered deltamethrin orally at 5mg/kg b.wt. The 4th and 5th groups were fed on standard ration containing Moringa and sage leaf powders at 5 and 2.5%, respectively, while the 6th and 7th groups were fed on standard ration containing Moringa leaves powder (5%) and received deltamethrin (10 and 5mg/kg b.wt., respectively), and the 8th and 9th groups were fed on standard ration containing sage leaves powder (2.5%) and received deltamethrin (10 and 5mg/kg b.wt., respectively). Feeding trial continued for 30 successive days. Results indicated the high nutritional values of Moringa and sage leaves. Although deltamethrin administration at its two doses did not affect the relative weights of sexual organs including testis, seminal vesicle gland and epididymis and prostate it caused significant reductions in both sperm cell count and motility while sperm abnormalities including bent head and detached tail were significantly increased. It can be concluded that supplementation of daily meals with dried Moringa and sage leaves are recommended to prevent from infertility resulted from exposure to deltamethrin.

INTRODUCTION

Flavonoids are a family of phenolic compounds that have many biological properties including hepatoprotective, antithrombotic, antibacterial, antiviral and anti carcinogenic activity. These physiological benefits thought to be due to their antioxidant activity and free radical scavenging properties (**Tiwari and Rao 2010**)

Moringa oleifera Lam (Family: *Moringaceae*) is a highly valued plant in tropical and subtropical countries where it is mostly cultivated. The leaves are highly nutritious, being a good source of β -carotene, riboflavin, nicotinic acid, folic acid, pyridoxine, protein, vitamins A, B, C and E, amino acids, various phenolic compounds and minerals (**Abbas et al ., 2018**) The scientific studies provide insights on the use of *M. oleifera* with different aqueous, hydroalcoholic, alcoholic, and other organic solvent preparations of different parts for therapeutic activities, that is, antibioid, antitumor, antioxidant, anti-inflammatory, cardio-protective, hepato-protective, neuro-protective, tissue-protective, and other biological activities with a high degree of safety. A wide variety of alkaloid and sterol, polyphenols and phenolic acids, fatty acids, flavanoids and flavanol glycosides, glucosinolate and isothiocyanate, terpene, anthocyanins etc. are believed to be responsible for the pragmatic effects. (**Ashok et al ., 2019**) *Moringa oleifera* is an edible medicinal plant used to fight malnutrition and health issues was highlighted in Africa. *M. oleifera* flowers, fruits and seeds from Guinea-Bissau were characterized for their nutritional composition and hydroethanolic and aqueous extracts were prepared to investigate the phenolic profiles and bioactivities. Seeds presented higher levels of proteins (~31 g/100 g dw), fat (~26 g/100 g dw) and flavan-3-ol derivatives, while carbohydrates, proteins, citric acid, and glycosylated flavonoids were abundant in fruits and flowers, these last samples also being rich in α -tocopherol (~18 mg/100 g dw). Some of the identified polyphenols had never been described in *M. oleifera*. (**Angela et al ., 2021**) *Moringa* can withstand both severe drought and mild frost conditions and hence widely cultivated across the world. With its high nutritive values every part of the tree is suitable for either nutritional or commercial purposes. The leaves are rich in minerals, vitamins and other essential phytochemicals. Extracts from the leaves are used to treat malnutrition, augment breast milk in lactating mothers. It is used as potential antioxidant, anticancer, anti-inflammatory, antidiabetic and antimicrobial agent (**Lakshmipriya et al ., 2016**).

Salvia officinalis L. (common sage) from the family *Lamiaceae* is a medicinal plant that is cultivated in various countries such as Canada and the USA.. Some studies indicated that some parts of the plant such as leaves and branches are rich in phenolic components that have antioxidant effects. The most important phenolic compounds that are found in this plant are phenolic acids such as caffeic, vanillic, ferulic, and rosmarinic acids, flavonoids such as luteolin, apigenin, and quercetin, as well as α - and β -thujone, 1, 8-cineole, camphor, carnosic acid, carnosol, rosmadial, manool, and volatile substances. (**Mahnaz et al ., 2020**) *Salvia officinalis* L. (*S. officinalis*, common sage) which is a medicinal plant well known for its reputation of

being a panacea and for its strong antioxidant properties attributed to its constitution in phenolic compounds (rosmarinic acid being the most representative (**Greer ., 2017**) Many researchers have investigated the common uses of sage and found different pharmacological functions such as anticancer, anti-inflammatory, antioxidant, antimicrobial, antimutagenic, hypolipidemic and hypoglycemic The alkaloids, carbohydrates, fatty acids, glycosidic derivatives, phenolic compounds, poly acetylenes, steroids, terpenes/terpenoids and waxes of *S. officinalis* were also identified, especially in leaf .(**Isam et al ., 2020**)

Deltamethrin (DEL)] is a type II highly active synthetic pyrethroid . The exposure to pyrethroids occurred through skin contact, inhalation, or food/water ingestion as well as the professional work, alimentation, and water are the main sources of exposure (**WHO 2016**). Oral exposure to DEL induces hepatotoxicity and nephrotoxicity in rats, increases lipid peroxidation levels, decreases antioxidant capacity, and affects the tumor necrosis factor- α , white blood cells, and erythrocyte count Moreover DEL decreases the sperm count, motility percentage and testosterone level, and the reproductive organ's weight and induces histological changes in the testicular tissues in rats.(**Nguemo et al. 2019 and Petrovici et al. 2020**).

Male infertility and impaired fecundity is a growing global health concern. An estimated 8-12% of couples have experienced some form of infertility, with causative factors in about 40% of the cases being traced back exclusively to the male partner of the couple.(**Olawale et al ., 2019**) Male sexual dysfunction composed of several problems associated with sperm concentration, motility and hormonal imbalance e.g., low testosterone level, which are caused by alcoholism, drug abuse, aging and cigarette smoking, anti-depressant drugs and exposure of toxic chemicals (**Othman et al., 2014**).

MATERIALS AND METHODS

Materials:

1- Plants: Moringa (*Moringa oleifera Lam*) and common sage (*Salvia officinalis L.*) were obtained as dry leaves from the Local Company for Herbs and Medicinal Plants, Cairo Governorate, Egypt.

2- Deltamethrin: Deltamethrin (oral LD₅₀ 200 mg/kg b.wt. of rat) **Ali et al., 2017** as one of pyrethroids was obtained from Central Agricultural Pesticides Laboratory. Dokki, Giza, Egypt.

3 -Animals: Fifty four adult normal male albino rats (Sprague Dawley strain) of an average body weight 150-180 g were obtained from the animal colony, Helwan farm, Vaccine and Immunity Organization, Ministry of Health, Cairo Governorate, Egypt.

4- Ration:All over the experimental period, rats were fed on standard ration supplying the essential macro- and micro nutrients. Ration was purchased from El-fagr CompanyCairo, Egypt.

5- Chemicals and kits:All required chemicals were obtained from El-Gomhoreya Company for trading drugs, chemicals and medical appliances, Cairo, Egypt. Kits used for biochemical determinations were obtained from Gama Trade Company for chemicals, Cairo, Egypt.

Experimental design:

The experiment was conducted in the Pharmacology Unit, Biochemistry, Toxicology and Food Deficiency Department, Animal Health Research Institute, Dokki, Giza. Rats were housed in wire cages in a room temperature maintained at $25 \pm 2^{\circ}\text{C}$ and kept under normal healthy conditions. Rats were fed the used ration for one week for adaptation. Meanwhile, water and ration were provided *ad libitum*. After that, rats were divided into 9 groups of 6 rats each as follows:

Group 1: Kept as negative (- ve) control and fed only on ration for 30 successive days.

Group 2: Kept as positive control 1, fed on ration and administered deltamethrin orally by gavage at dose (10mg/kg b.wt.) for 30 successive days.

Group 3: Kept as positive control 2, fed on ration and administered deltamethrin orally by gavage at dose (5mg/kg b.wt.) for 30 successive days.

Group 4:Fed on ration mixed with Moringa leaves powder at concentration 5% for 30 successive days.

Group 5:Fed on ration mixed with sage leaves powder at concentration 2.5% for 30 successive days.

Group 6: Fed on ration mixed with Moringa leaves powder at concentration 5% and administered deltamethrin orally by gavage at dose (10mg/kg b.wt.)for 30 successive days.

Group 7:Fed on ration mixed with Moringa leaves powder at concentration 5% and administered deltamethrin orally by gavage at dose (5mg/kg b.wt.)for 30 successive days.

Group 8:Fed on ration mixed with sage leaves powder at concentration 2.5% and administered deltamethrin orally by gavage at dose (10mg/kg b.wt.) for 30 successive days.

Group 9:Fed on ration mixed with sage leaves powder at concentration 2.5% and administered deltamethrin orally by gavage at dose (5mg/kg b.wt.)for 30 successive days.

Methods:

1- Chemical composition of Moringa and sage leaves:

Moisture content, crude protein and ash content were determined according to the described by AOAC (1995), the fat content according to Horwitz (1980), crude protein Less (1975), and vitamin E according to published procedure Egyptian pharmacopeia (1984). The chemical composition of tested plants was recorded in table(1) The record results are in agreement with those found by (Halaby et al., 2015)

2- Effect of Moringa and sage leaves versus deltamethrin on male rat fertility:

By the end of the experiment, rats were fasted overnight and sacrificed. Relative weights of sexual organs were calculated and spermatozoa examination was done.

- 1- Calculation of relative weights of sexual organs
- 2- Epididymal spermatozoal examination
- 3- Progressive motility by (Bearden and Fluquery, 1980)
- 4- Sperm cell concentration
- 5- Epididymal sperm abnormalities

3 - Estimation of thyroid hormones:

- 1 - Serum total T₃ (triiodothyronine)
- 2 - Serum total T₄ (thyroxine): Total T₄ was determined in serum according to the method described by Kricka, (2000)
- 3 - Serum thyroid stimulating hormone (TSH): Thyroid stimulating hormone (TSH) was determined in serum according to the method described by Spencer *et al.*, (1995)
- 4- Estimation of testosterone: Testosterone was determined in serum according to the method described by Kicman, (1995)

Statistical analysis:

Statistical analysis was carried out using the programme of Statistical Package for the Social Sciences (SPSS), PC statistical software (Version 20; Untitled – SPSS Data Editor).

The results were expressed as mean \pm standard deviation (mean \pm SD). Data were analyzed using one way (ANOVA) test. The differences between means were tested for significance using Duncan test at $p < 0.05$. Independent T test was also used to determine the statistical difference between two means (Snedecor and Cochran, 1989).

RESULT AND DISCUSSION

The main objective of this study was to investigate the efficiency of Moringa (*Moringa oleifera* L.) and common sage (*Salvia officinalis* L.) leaves as protective agents on deltamethrin-induced morphological and functional changes in gonads of male albino rats. Deltamethrin is a synthetic pyrethroid insecticide used to control numerous insect pests of field crops, potted plants, and ornamentals. (National Pesticide Information Center, 2012). One of the unwanted side effects of

deltamethrin is decreased and abnormalized spermatogenesis which resulted finally in infertility (Yu *et al.*, 2014). Male infertility is a multi factorial disease process with a number of potential contributing causes (Nazni, 2014). Sexual function greatly affects individual's quality of life the normal male sexual response cycle consists of five phases: libido, erection, ejaculation, orgasm and detumescence subsequently. Any problem which affects satisfaction is considered sexual dysfunction (Abbas *et al.*, 2016). Male sexual dysfunction is growing world widely. Sexual dysfunction has many etiological factors including various physical and psychological conditions

The male accounts for 30%–50% of entire infertility causes 25% of them are unknown causes, without knowing the exact etiology, targeted (management is not applicable, rising the use of empiric treatment in present conventional medicine without sufficient scientific evidence (Bassam and. Alahmadi 2020)

Effects of Moringa and sage leaves on fertility markers

1- Effects on the relative weights of sexual organs:

Effects of feeding on ration mixed with Moringa and sage leaves at concentrations 5 and 2.5%, respectively for 30 successive days with or without oral administration of deltamethrin at doses (10&5 mg \kg b.wt.) on the relative weights of sexual organs were recorded **in table (2)**.Unexpectedly, oral administration of deltamethrin at the two used doses (10 and 5 mg/kg b. wt.) resulted in no significant differences in the relative weights of all studied sexual organs compared with control –ve group. Also, feeding on ration mixed with either 5% Moringa or 2.5% sage leaves could not induce any significant changes in the relative weights of testis, epididymis and prostate in the groups received either the high dose or the low dose of deltamethrin, although the decrease noticed in relative testis weight These results are agreement with Samar *et al.*, (2019) demonstrated that moringa alone induced a stimulatory effect on the expression of steroidogenic and LHR genes. It restored the weight of reproductive organs to the control level however the recovery in sperm count, motility, abnormalities, percentage of alive sperm, testosterone, and MDA level are still comparable with the control level. Similar findings were also reported at the histological structure of the testes, epididymis, and accessory sex glands . As for the relative weight of seminal vesicle glands, 5% Moringa leaves induced a significant decrease only in the group administered the low dose of deltamethrin, while it had no significant effect on the group received the high dose. On the other hand, 2.5 % sage leaves were more potent and resulted in significant reductions in the relative weight of S.v. of the two deltamethrin administered groups and disagree with (Nguemo *et al.* 2019 and Petrovici *et al.* 2020).. Similarly Desai *et al.*, (2016) demonstrated that tissue weight of testis, cauda epididymis and seminal vesicle of both low dose (3 mg/kg b. wt.) and high dose (6 mg/kg b. wt.) of deltamethrin -treated mice after 45 days recorded a significant fall ($P < 0.05$ or $P < 0.01$) as compared to control mice. These result are agree with (Abarikwu *et al.* , 2017 and Bassam and. Alahmadi 2020) found that Moringa has the capability to inhibit damage of the

testis. As testicular toxicity induced by mercury on male rat could be prevented by administration of 2 mL/kg⁻¹ body weight of moringa oil orally .It also have positive result on testicular weight, proved by using Moringa leaf extract at 500 mg/kg⁻¹ orally for 60 days .

2 Effects on epididymal sperm characters:

Oral administration of deltamethrin at the two used doses (10 and 5 mg/kg b. wt.) resulted in significant reductions in sperm cell motility and count compared with control –ve group, while sperm abnormalities, including detached head and coiled tail, were significantly increased.on epididymal sperm characters were recorded in table (3)These results are in line with (Desai et al.,2016) and (Abarikwu et al ., 2017) .It could be noticed that rats feed on ration mixed with either 5% Moringa or 2.5% sage leaves protective effect on reproductive system as they improved cell count and motility significantly in the groups received either the high dose or the low dose of deltamethrin. Moringa and sage feeding in the present study showed a protective effect on reproductive system as they improved cell count and motility significantly in the groups received either the high dose or the low dose of deltamethrin. As for sperm abnormalities, 2.5 % sage leaves was more potent and resulted in significant reductions in the two deltamethrin –administered groups, while Moringa leaves positively affected only the group received the high dose. These results are in harmony with many studies mentioned in the literature. For example, Navodita and Varma, (2014) found that hyperglycemic mice fed with 200mg/ kg body weight of Moringa leaf powder showed significant increases in sperm count and mobility, while sperm mortality decreased significantly. Regarding sage leaves effect, the present results are in agreement with Al-Chalabi et al., (2016) and Ahmed et al., (2017). Ahmadi et al., (2013) indicated that *Salvia officinalis* extract (150 and 200 mg/kg) increased seminiferous tubule diameter and number of sperms in tubule tunnel ($P<0.01$), i.e. it leads to increase spermatogenesis. (Sadek *et al.*, 2017) suggested that the aqueous extract of *Moringa oleifera* seeds may improve male sexual behavior due to an observed increase in libido, sperm count, mounting frequency, intromission frequency, and ejaculation latency with reduction in mounting latency, intromission latency, and post ejaculatory interval.

Effects of Moringa and sage leaves on thyroid and testosterone hormones:

As for thyroid hormones and testosterone the present findings revealed that oral administration of deltamethrin at the two used doses resulted in no significant increases in T₃, T₄ and testosterone levels in serum in spite of a significant decrease noticed in TSH level compared with –ve control groupwere record in **table (4)** .Thus it looks like that the type of thyroid disease induced by deltamethrin, in the present study is hyperthyroidism and not hypothyroidism. These results are agree with (**Shui et al., 2018**) showed that deltamethrin decreased the relative thyroid weight in 0.3 and

1 mg/kg/day in female but not in male rats. Although the histology and several parameters of thyroid were not affected, the decreased relative weight exhibited underlying meaning. The novel finding is that deltamethrin decreased thyroxine (T₄), triiodothyronine (T₃), and thyroid-stimulating hormone (TSH) in the female rats. In contrast, deltamethrin increased T₃ and TSH but not in T₄ in male rats. We inferred that deltamethrin disrupts thyroid hormone and might be related to estrogen receptor agonist.

In the present study feeding on ration mixed with 2.5% sage caused insignificant decrease in the levels of T₃ and T₄ in the groups received either the high dose or the low dose of deltamethrin. Sage feeding also could increase and normalize TSH level in the group received the high dose of deltamethrin, while it could decrease and normalize testosterone level in the group received the low dose. It was suggested that the antioxidant activity of sage is the mechanism by which it induced these useful effects. This suggestion is in agreement with **Ahmed et al., (2017)** found that chlorpyrifos - and methomyl -administered rats exhibited a significant increase in testis and heart lipid peroxidation as well as a significant decrease in reduced glutathione content and superoxide dismutase, glutathione peroxidase and glutathione-S-transferase activities. Concomitant supplementation with *Salvia officinalis* ethanolic extract markedly prevented chlorpyrifos- and methomyl-induced biochemical and histopathological alterations in rats through potentiation of the antioxidant defense system. Unlike sage in deltamethrin -received groups in the present study, Moringa leaves induced no significant influences on thyroid hormones and testosterone levels in serum in spite of the high content of antioxidants contained within. This may be due to low experimental period, i.e. more time was needed to exhibit the effectiveness of Moringa leaves in improving thyroid functions and serum testosterone level. **(Olawale et al ., 2019)** found that The methanol fraction of *Moringa* seeds significantly decreased testosterone, luteinizing hormone, sperm motility, and sperm count of treated rats when compared with controls. The hexane fraction of *Moringa* seeds had no effect on sex hormones or sperm profiles. Both methanol and hexane fractions significantly increased superoxide dismutase and catalase levels, while malondialdehyde levels decreased significantly.

Table (1): Chemical composition of the used Moringa and sage leaf samples

Nutrients	Nutritional value (/100g dry weight)	
	Moringa	Sage
Energy (kcal)	326.3	378.5
Protein (g)	18.74	9.54
Total fat (g)	2.86	12.98
Dietary ash (g)	12.2	11.6
Carbohydrates (g)	56.4	55.88
Crude fiber (g)	11.4	12.8
Moisture (g)	9.8	10
Aflatoxin	None	None
Ca (mg)	3200	2980
P (mg)	1000	1200
Zinc (mg)	2.70	4.50
Vit. A μ g RAE	1250	1800
Vit. E (mg)	40	7.55

- Kcal=kilocalories, g= grams, mg= milligrams. μ g RAE= micrograms retinol activity equivalents.

Table [2]: Effects of Moringa and sage leaves on the relative weights of sexual organs in normal and deltamethrin administered rats (n=5)

Groups	Dose	Testis(%)	S.v(%)	Epididymis(%)	Prostate(%)
Control (-ve)		0.74±0.09 ^b	0.51±0.04 ^a	0.29±0.03 ^b	0.11±0.02 ^a
Deltamethrin	10	1.00±0.07 ^{ab}	0.57±0.04 ^a	0.28±0.05 ^b	0.14±0.02 ^a
Deltamethrin	5	0.99±0.01 ^{ab}	0.50±0.04 ^a	0.37±0.09 ^{ab}	0.15±0.01 ^a
Moringa	5%	1.05±0.04 ^a	0.66±0.07 ^a	0.33±0.07 ^b	0.15±0.04 ^a
Sage	2.5%	1.07±0.04 ^a	0.55±0.08 ^a	0.36±0.28 ^{ab}	0.14±0.04 ^a
Moringa + Delt.	5+10	0.85±0.19 ^{ab}	0.55±0.04 ^a	0.31±0.04 ^b	0.16±0.01 ^a
Moringa + Delt.	5+5	0.94±0.07 ^{ab}	0.20±0.04 ^b	0.42±0.02 ^{ab}	0.17±0.02 ^a
Sage + Delt.	2.5+10	0.84±0.03 ^{ab}	0.26±0.08 ^b	0.50±0.04 ^{ab}	0.13±0.03 ^a
Sage + Delt.	2.5+5	0.89±0.06 ^{ab}	0.25±0.03 ^b	0.50±0.04 ^{ab}	0.16±0.01 ^a

- Values are expressed as mean ± S. D.
- Significance is expressed at p<0.05 using one way ANOVA test and Duncan test.
- Values which have different letters in each column differ significantly, while the difference among those with similar letters completely or partially is not significant.

Table [3]: Effects of Moringa and sage leaves on epididymal sperm characters in normal and deltamethrin –administered rats (n = 5)

Groups	Dose	Motility (%)	Count (10 ⁶ / epididymis)	Abnormality (%)
Control (-ve)		88.80±0.97 ^b	68.00±0.71 ^a	5.06±0.15 ^e
Deltamethrin	10	35.00±2.74 ^f	16.20±0.58 ^f	28.08±1.32 ^a
Deltamethrin	5	48.00±2.55 ^e	23.00±1.14 ^e	20.60±0.52 ^c
Moringa	5%	94.00±1.00 ^a	66.20±3.42 ^a	4.76±0.27 ^e
Sage	2.5%	87.00±1.22 ^b	65.60±0.40 ^a	4.76±1.19 ^e
Moringa +Delt.	5+10	61.00±1.00 ^c	37.20±1.16 ^{cd}	25.70±1.09 ^b
Moringa +Delt.	5+5	65.80±1.77 ^c	59.00±1.87 ^b	18.52±0.88 ^{cd}
Sage +Delt.	2.5+10	52.00±1.22 ^d	33.00±1.22 ^d	25.18±0.72 ^b
Sage +Delt.	2.5+5	56.00±1.87 ^d	41.00±1.87 ^c	17.68±0.61 ^d

- Values are expressed as mean ± S. D.
- Significance is expressed at p<0.05 using one way ANOVA test and Duncan test.
- Values which have different letters in each column differ significantly, while the difference among those with similar letters completely or partially is not significant.

Table (4): Effects of Moringa and sage leaves on thyroid and testosterone hormones in serum of normal and deltamethrin administered rats (n=5)

Groups	Dose	T ₃ (ng/ml)	T ₄ (ug/dl)	TSH (μIU/ml)	Testosterone (ng/ml)
Control (-ve)		0.81±0.01 ^a	6.66±0.08 _{ab}	0.0020±0.0003 _a	0.06±0.01 ^{bc}
Deltamethrin	10	0.87±0.05 ^a	6.88±0.59 _a	0.0012±0.0002 _b	0.08±0.00 ^{ab}
Deltamethrin	5	0.84±0.03 ^a	6.80±0.24 _a	0.0010±0.0000 _b	0.08±0.01 ^{ab}
Moringa	5%	0.84±0.06 ^a	6.38±0.14 _{ab}	0.0010±0.0000 _b	0.09±0.01 ^{ab}
Sage	2.5%	0.71±0.02 ^a	5.75±0.10 _{bc}	0.0010±0.0000 _b	0.09±0.01 ^{ab}
Moringa + Delt.	5+10	0.79±0.03 ^a	5.98±0.15 _{abc}	0.0012±0.0002 _b	0.07±0.01 ^{ab}
Moringa + Delt.	5+5	0.73±0.09 ^a	6.11±0.62 _{abc}	0.0012±0.0002 _b	0.08±0.01 ^{ab}
Sage + Delt.	2.5+10	0.77±0.03 ^a	6.14±0.17 _{abc}	0.0022±0.0001 _a	0.08±0.01 ^{ab}
Sage + Delt.	2.5+5	0.81±0.04 ^a	6.23±0.16 _{abc}	0.0008±0.0002 _b	0.04±0.01 ^c

- Values are expressed as mean ± S. D.
- Significance is expressed at p<0.05 using one way ANOVA test and Duncan test.
- Values which have different letters in each column differ significantly, while the difference among those with similar letters completely or partially is not significant.

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