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## Research Article

# Pyridoxine Improving Effect on Yield, Chemical and Nutritional Value of Egyptian Clover Plant

<sup>1</sup>M.F. El Karamany, <sup>2</sup>Mervat Sh. Sadak, <sup>1</sup>Gehan Sh. Bakhom, <sup>3</sup>Hamed A.A. Omer and <sup>1</sup>Bakry A. Bakry

<sup>1</sup>Department of Field Crops, Agricultural and Biological Research Institute, National Research Centre, 33 El-Bohouth Street, P.O. Box 12622, Dokki, Giza, Egypt

<sup>2</sup>Department of Botany, Agricultural and Biological Research Institute, National Research Centre, 33 El-Bohouth Street, P.O. Box 12622, Dokki, Giza, Egypt

<sup>3</sup>Department of Animal Production, Agricultural and Biological Research Institute, National Research Centre, 33 El-Bohouth Street, P.O. Box 12622, Dokki, Giza, Egypt

## Abstract

**Background and Objective:** Vitamins are natural substances that are important in tiny quantities to sustain the normal growth and development of the plant. Thus this study was carried out to study the physiological effect of pyridoxine on increasing yield quantity and quality of Egyptian clover plant. **Materials and Methods:** In sandy soil at the Research and Production Station, National Research Centre, Nubaria Province, Behaira Governorate, two field experiments were carried out during two consecutive winter seasons to study the effects of pyridoxine treatment on the yield and nutritional value of Egyptian clover. **Results:** Spraying Egyptian clover with pyridoxine significantly increased yield attributes, crude protein, Ash contents, however, crude fibre content decreased by 50 or 75 mg L<sup>-1</sup>. Pyridoxine levels significantly decreased nitrogen, free extract contents. Pyridoxine with 100 mg L<sup>-1</sup> significantly increased gross, digestible, metabolizable and net energy, total digestible nutrients, digestible crude protein, neutral detergent fibre, acid detergent fibre, acid detergent lignin and cellulose contents in comparison with control and the other treatments. Cut stages affect the significantly chemical composition and their contents of both energetic and nutritive values and cell wall constituents. Moreover, there were significant interactions between pyridoxine supplementation and berseem cut stages on chemical composition and their contents of energetic and nutritive values and cell wall constituents. Also, photosynthetic pigments, carbohydrates constituents, indole acetic acid, phenolic and flavonoids contents were increased gradually with increasing pyridoxine concentrations. **Conclusion:** In conclusion, different pyridoxine treatments have a promotive effect on increasing the forage yield of the berseem plant in addition to its nutritive values.

**Key words:** Pyridoxine, Egyptian clover, crop yield, chemical analysis, cell wall constituents, energetic, nutritive values, IAA, phenols

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**Corresponding Author:** Mervat Sh. Sadak, Department of Botany, Agricultural and Biological Research Institute, National Research Centre, 33 El-Bohouth Street, P.O. Box 12622, Dokki, Giza, Egypt

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**Competing Interest:** The authors have declared that no competing interest exists.

**Data Availability:** All relevant data are within the paper and its supporting information files.

## INTRODUCTION

Egyptian clover (*Trifolium alexandrinum* L.) is an annual, cool-season forage crop grown in diverse conditions of Egypt. It gives several cuttings during its growing season and supplies nutritious and juicy forage for animals<sup>1</sup>. Normally four to six cuttings of berseem are taken<sup>2</sup>. It is used as animal feed either green or in hay form when seasonal conditions permit<sup>3,4</sup>. It is famous for farmers due to its rapid growth, an excessive wide variety of harvests and clean forage manufacturing with correct high-satisfactory and quantity<sup>5,6</sup>. The production rate of Egyptian clover depends on sowing date, climatic conditions, soil fertility, shrub height, number of crops and variety<sup>2,3</sup>. Therefore, one of the most important goals of Egyptian crops is to increase forage production, especially Egyptian clover, by maintaining its properties as a vegetation cover and soil fertility<sup>4</sup>. It also plays an important role in increasing the intake of dairy products and animal protein. In the same context, Arif *et al.*<sup>7</sup> cleared that Egyptian clover (*Trifolium* spp.) is one of the most important forage legumes and is known as the Forage King. Often used as green feed for all livestock. Dried Egyptian clover is also an important poultry feed. Due to its desirable properties, it has been suggested that it may be an inexpensive additional source of protein in cattle feed<sup>8</sup>.

Salem *et al.*<sup>9</sup> also reported that Egyptian clover (*Trifolium alexandrinum* L.) can grow as the first forage plant under Egyptian conditions. The cultivated area is approximately 1.07 million ha/year. In addition, Egyptian clover is an important forage plant because it contains high levels of protein, phosphorus, calcium and various amino acids (valine, leucine, methionine, lysine, tryptophan, arginine and others) as well as 0.75 kg of energy units, provided in every kilogram of hay, so clover improves the structure of the soil. Moreover, the presence of bacteria in the roots of clover increases nitrogen in the soil (100-150 kg of nitrogen is produced per hectare of soil, which is equivalent to 400-500 kg of ammonium chloride). In addition, Egyptian clover plays an important role not only in reducing soil erosion and salt formation but also in cleaning the soil from weeds<sup>3</sup>.

Egypt is suffering from the loss of cropland due to erosion and desertification. Therefore, it is very important to increase the area of the agricultural land. The newly reclaimed sandy soil on the outskirts of the Nile has received a lot of attention in Egypt. Reclaimed sandy soils are affected by a combination of nutrient deficiencies and lack of available water as well as biological stresses such as temperature fluctuations and increased lighting<sup>10</sup>.

So, to increase the resistance of plants to these destructive conditions, unusual methods are used. These strategies include selecting resistant varieties, using optimal growing methods and using natural compounds (amino acids, vitamins and antioxidants) through various treatments such as soaking seeds or treating leaves. Therefore, the use of various substances or vitamins that control growth has an important effect on the growth and yield of plants and affects various biochemical and physiological processes of plants. Amino acids are one of the precursors of these growth-promoting substances<sup>11</sup>.

Vitamins are natural substances that are essential in trace quantities to sustain the normal growth and proper development of all organisms and they function as coenzyme systems and thus play an essential role in the regulation of metabolism. Vitamins are known as limiting factors in plant development<sup>12</sup>. The diverse treatment of vitamins led to an effective role of plant bioregulators, which then influence various biochemical processes and protect the plant from the negative influences of abiotic stress<sup>13</sup>. Pyridoxine (vitamin B<sub>6</sub>) is an essential coenzyme which incorporated in a wide range of physiological processes, among them glycogen metabolism and biosynthesis of amino acids. Pyridoxine can act as a co-enzyme for numerous metabolic enzymes<sup>14</sup> and it is shown to be a potent antioxidant<sup>15,16</sup>. It improved the efficiency of photosynthetic carbon reactions and increased dry matter production. In the meantime, treatment of different plants with pyridoxine enhanced cell division, improved growth and differentiation and increased nutrient uptake<sup>17</sup>. In seedling growth, exogenous treatments with optimal pyridoxine concentrations were beneficial via increasing availability of water and nutrient. Pyridoxine is highly recommended for use in various plants. It has been established that pyridoxine enhances the growth of the root system which helps in higher nutrient uptake and leads to higher economic yield<sup>18</sup>.

So, the objective of this search is to investigate the influence of pyridoxine in different amounts (0, 50, 75 and 100 mg L<sup>-1</sup> of water) of supplementation on the yield of the crop in different stages of cutting forage of Egyptian clover, which is cultivated under sandy soil for its increase the harvest as an added value in animal feed and improve its chemical, physiological, biochemical processes, composition and nutritional values.

## MATERIALS AND METHODS

**Study area:** The study was conducted in co-operation work among Department of Field Crops, Institute of Agriculture and Biological Researches, National Research Center, Dokki, Cairo,

Egypt and Department of Animal Production, Institute of Agriculture and Biological Researches, National Research Center, Dokki, Cairo, Egypt.

The purpose of this study was to influence the amount of Egyptian clover (crop yield) and chemicals by including pyridoxine in various amounts (0, 50, 75 and 100 mg L<sup>-1</sup> water) of supplements. It was to find out investigate composition at various cutting stages (4 cuts).

In the winter of 2019/2020 and 2020/2021, two field experiments were conducted at the Research and Production Station of National Research Centre (NRC), Al-Nubaria District, Al Behaira Governorate, Egypt. The experimental soil before sowing was analyzed according<sup>19</sup>. The analysis of the experimental soil was: Soil texture: Sand 91.2%, Silt 3.7%, Clay 5.1%, pH 7.3, Organic matter 0.3%, CaCO<sub>3</sub> 1.4%, E.C. dS/m 0.3, Soluble N, ppm 8.1, Available P 3.2 ppm, Exchangeable K, 20 ppm.

Experimental soil was ploughed twice and divided into plots 3×7 m, then made rows 20 cm<sup>2</sup> between. Egyptian clover cultivar (Meskawy) was inoculated with the appropriate (*Rhizobium trifolii*) in a commercial product produced by the Ministry of Agriculture, Egypt.

The recommended agricultural practices were applied. Pre-sowing, 150 kg/feddan of calcium super-phosphate (15.5% P<sub>2</sub>O<sub>5</sub>) was applied to the soil. Nitrogen was applied after emergence in the form of ammonium nitrate 33.5% at a rate of 75 kg/feddan in five equal doses before the 1st, 2nd, 3rd and 4th irrigation.

Potassium sulfate (48.52% K<sub>2</sub>O) was added in two equal doses of 50 kg/feddan, before the 1st and 3rd irrigations. Irrigation was carried out using the new sprinkler irrigation system where water was added every 5 days.

The applied substance pyridoxine used in the present study was supplied from sigma chemical.

The plants were sprayed twice with pyridoxine at (50, 75 or 100 mg L<sup>-1</sup>) while control plants were sprayed with distilled water during vegetative growth at 30 and 45 days after sowing.

Four cuts were taken from each of the two seasons. The first cut was obtained 60 days post-seeding date, the second cut was obtained after 50 days from the first one, while the third one was taken after 40 days from the second cut and the fourth was taken after 40 days from the third cut.

**Fresh forage yields:** Fresh forage yield of Egyptian clover determined in m<sup>2</sup> for each of the subsequent four cuts, in each experimental plot recorded and estimated in ton/feddan. This was done for each of the two growing seasons.

**Biochemical determinations:** Photosynthetic pigments (chlorophyll a and b, carotenoids and total pigments) in fresh leaves were determined as the method described by Lichtenthaler and Buschmann<sup>20</sup>, total carbohydrates was determined according<sup>21</sup>, total soluble sugars were extracted by the method<sup>22</sup> and extracted by the method<sup>21</sup>, polysaccharides were determined according<sup>21</sup>, phenolic content was measured as the method described<sup>23</sup> and flavonoids contents were determined by the method<sup>24</sup>.

Different samples of unsprayed Egyptian clover and sprayed Egyptian clover that sprayed by pyridoxine solution at different levels mentioned above were collected at different cuts and chemical analyses were determined for their contents of moisture, Dry Matter (DM), Organic Matter (OM), Crude Protein (CP), Crude Fibre (CF), Ether Extract (EE), Nitrogen-Free Extract (NFE) and Ash using methods that described<sup>25</sup>.

On other hand, cell wall constituents include Neutral Detergent Fibre (NDF), Acid Detergent Fibre (ADF), Acid Detergent Lignin (ADL), hemicellulose and cellulose were evaluated in both unsprayed Egyptian clover and sprayed Egyptian clover as the method<sup>26,27</sup>.

Energetic values composed of Gross Energy (GE), Digestible Energy (DE), Metabolizable Energy (ME) and Net Energy (NE), in addition to nutritive values of both Total Digestible Nutrients (TDN) and Digestible Crude Protein (DCP) were also calculated according to different equations that concluded by NRC<sup>28</sup>.

**Analytical procedures:** Chemical analysis of unsprayed Egyptian clover and Egyptian clover includes moisture, Ash, Crude Protein (CP), Crude Fibre (CF) and Ether Extract (EE) contents were determined according to Talreja *et al.*<sup>25</sup>.

Crude protein determination involved the use of routine Kjeldahl nitrogen assay (N×6.25).

Meanwhile, Nitrogen-Free Extract (NFE) or carbohydrate content was determined by the difference using the following equation:

$$\text{NFE content} = 100 - (\text{Moisture} + \text{CP} + \text{CF} + \text{EE} + \text{Ash})$$

On the other hand, cell wall constituents including Neutral Detergent Fiber (NDF), acid detergent fibre and Acid Detergent Lignin (ADL) were determined according to Samreen *et al.*<sup>26</sup> and Vishwakarma and Dubey<sup>27</sup>. However, hemicellulose and cellulose contents were calculated by difference as follows:

- Hemicellulose = NDF-ADF
- Cellulose = ADF-ADL

Gross energy (Kcal Kg<sup>-1</sup> DM) was calculated according to Liu *et al.*<sup>29</sup>.

where, each g crude protein = 5.65 Kcal, g fat = 9.40 Kcal and g (crude fibre and carbohydrate) = 4.15 Kcal.

Digestible energy (Kcal kg<sup>-1</sup> DM) was calculated according to NRC<sup>28</sup>.

Where:

$$\text{Digestible Energy (DE)} = \text{Gross energy} \times 0.76$$

Metabolizable energy (Kcal kg<sup>-1</sup> DM) was calculated according to NRC<sup>28</sup>.

Where:

$$\text{Metabolizable Energy (ME)} = \text{Digestible energy} \times 0.82$$

Net energy (Kcal kg<sup>-1</sup> DM) was calculated according to NRC<sup>28</sup> as follows:

$$\text{Net Energy (NE)} = \text{Metabolizable energy} \times 0.56$$

Total digestible nutrients (%) was calculated according to NRC<sup>28</sup>.

Where:

$$\text{Total digestible nutrients (\%)} = \frac{\text{Digestible energy}}{44.3}$$

Digestible crude protein (%) was calculated according to NRC<sup>28</sup>.

Where:

$$\text{Digestible crude protein (\%)} = 0.85 X_1 - 2.5$$

where, X<sub>1</sub> = Crude protein (%) on DM basis.

**Statistical analysis:** Except for data of indole acetic acids the others collected data of chemical composition that includes (Moisture, Dry Matter (DM), Organic Matter (OM), Crude Protein (CP), Crude Fibre (CF), Ether Extract (EE), Nitrogen-Free Extract (NFE) and Ash), cell wall constituents includes (Neutral Detergent Fibre (NDF), Acid Detergent Fibre (ADF), Acid Detergent Lignin (ADL), Hemicellulose and Cellulose), energetic values include (Gross Energy (GE), Digestible Energy (DE), Metabolizable Energy (ME) and Net Energy (NE)) and

nutritive values includes Total Digestible Nutrients (TDN) and Digestible Crude Protein (DCP), fresh forage yields, photosynthetic pigments includes (chlorophyll a and b, carotenoids and total pigments), total carbohydrates, total soluble sugars, polysaccharides, phenolic and flavonoids contents were statistically analyzed as two factors-factorial analysis of variance using the general linear model procedure of Salkind<sup>30</sup> was used to examine the significance between means.

The following model was used as the following:

$$Y_{ijk} = \mu + L_i + C_j + (LC)_{ij} + e_{ijk}$$

Where:

Y<sub>ijk</sub> = Observation

μ = Overall mean

L<sub>i</sub> = Effect of levels of pyridoxine solution sprayed for i = 1-4, 1 = 0 pyridoxine, 2 = 50 mg pyridoxine/L water, 3 = 75 mg pyridoxine/L water and 4 = 100 mg pyridoxine/L water

C<sub>j</sub> = Effect of Egyptian Clover (EC) Cut stages (C) for j=1-4, 1= First cut stage of EC, 2 = Second cut stage of EC, 3 = Third cut stage of EC and 4 = Fourth cut stage of EC

(LC)<sub>ij</sub> = Interaction between pyridoxine supplementation levels and EC cut stages

e<sub>ijk</sub> = Experimental error

Meanwhile, data of indole was statistically analyzed as one-way analysis of variance using the general linear model procedure of Boghdady<sup>31</sup> that used to examine the significance between means, using the following model:

$$Y_{ij} = \mu + L_i + e_{ij}$$

Where:

Y<sub>ij</sub> = Observation

μ = Overall mean

L<sub>i</sub> = Effect of levels of pyridoxine solution sprayed for i = 1-4, 1 = 0 pyridoxine, 2 = 50 mg pyridoxine/L water, 3 = 75 mg pyridoxine/L water and 4 = 100 mg pyridoxine/L water

e<sub>ij</sub> = Experimental error

## RESULTS

**Changes in fresh forage yield:** Table 1, 2 cleared that, sprayed Egyptian clover forage with various pyridoxine levels (50, 75 or 100 mg L<sup>-1</sup>) realized a significantly (p<0.05) increasing in yield quantity compared with control plants. Meanwhile, cut stages

Table 1: Main consequences of nutritional remedies on forage yield

Items	Pyridoxine treatment levels (mg L <sup>-1</sup> )				SEM
	0	50	75	100	
Forage yield (ton/feddan)	2.946 <sup>d</sup>	3.117 <sup>c</sup>	3.701 <sup>b</sup>	4.539 <sup>a</sup>	0.115
Egyptian clover cut stages					
	First cut	Second cut	Third cut	Fourth cut	SEM
Forage yield (ton/feddan)	2.968 <sup>d</sup>	3.530 <sup>c</sup>	3.870 <sup>a</sup>	3.786 <sup>b</sup>	0.115

<sup>a-d</sup>Means in the same row within each treatment having different superscripts differ significantly (p<0.05) and SEM: Standard error of the mean

Table 2: Interactions between pyridoxine treatment levels and Egyptian clover forage cut stages on forage yield

Treatments	Pyridoxine treatment (mg L <sup>-1</sup> )			
	0	50	75	100
First cut	2.223 <sup>n</sup>	2.350 <sup>m</sup>	3.123 <sup>k</sup>	4.424 <sup>d</sup>
Second cut	3.122 <sup>k</sup>	2.867 <sup>l</sup>	3.600 <sup>q</sup>	4.530 <sup>c</sup>
Third cut	3.320 <sup>j</sup>	3.400 <sup>h</sup>	4.100 <sup>e</sup>	4.660 <sup>b</sup>
Fourth cut	3.120 <sup>k</sup>	3.300 <sup>i</sup>	3.982 <sup>f</sup>	4.740 <sup>a</sup>
SEM	0.115			

<sup>a-n</sup>Means in the same row having different superscripts differ significantly (p<0.05) and SEM: Standard error of the mean

Table 3: Chemical analysis of pyridoxine treatment on energetic and nutritive values and cell wall constituents

Items	Pyridoxine treatment levels mg L <sup>-1</sup> water				SEM
	0	50	75	100	
<b>1-Chemical analysis</b>					
Moisture	9.39 <sup>b</sup>	9.10 <sup>c</sup>	10.16 <sup>a</sup>	10.14 <sup>a</sup>	0.26
Dry Matter (DM)	90.61 <sup>b</sup>	90.90 <sup>a</sup>	89.84 <sup>c</sup>	89.86 <sup>c</sup>	0.26
<b>Chemical analysis on DM basis</b>					
Organic Matter (OM)	88.01 <sup>a</sup>	86.81 <sup>c</sup>	87.52 <sup>b</sup>	88.03 <sup>a</sup>	0.18
Crude Protein (CP)	17.09 <sup>d</sup>	17.83 <sup>c</sup>	18.53 <sup>b</sup>	18.64 <sup>a</sup>	0.15
Crude Fiber (CF)	21.04 <sup>b</sup>	20.45 <sup>d</sup>	20.88 <sup>c</sup>	22.04 <sup>a</sup>	0.45
Ether Extract (EE)	3.03 <sup>b</sup>	2.31 <sup>d</sup>	2.85 <sup>c</sup>	3.46 <sup>a</sup>	0.14
Nitrogen-Free Extract (NFE)	46.85 <sup>a</sup>	46.22 <sup>b</sup>	45.26 <sup>c</sup>	43.89 <sup>d</sup>	0.48
Ash	11.99 <sup>c</sup>	13.19 <sup>a</sup>	12.48 <sup>b</sup>	11.97 <sup>c</sup>	0.18
<b>2-Energetic and nutritive values</b>					
Gross Energy (GE)	4068 <sup>b</sup>	3992 <sup>d</sup>	4059 <sup>c</sup>	4114 <sup>a</sup>	10.83
Digestible Energy (DE)	3092 <sup>b</sup>	3034 <sup>d</sup>	3085 <sup>c</sup>	3127 <sup>a</sup>	8.22
Metabolizable Energy (ME)	2535 <sup>b</sup>	2488 <sup>d</sup>	2530 <sup>c</sup>	2564 <sup>a</sup>	6.73
Net Energy (NE)	1420 <sup>b</sup>	1393 <sup>d</sup>	1417 <sup>c</sup>	1436 <sup>a</sup>	3.79
<b>3-Nutritive values (%)</b>					
Total Digestible Nutrients (TDN)	69.79 <sup>b</sup>	68.48 <sup>d</sup>	69.63 <sup>c</sup>	70.59 <sup>a</sup>	0.19
Digestible Crude Protein (DCP)	12.03 <sup>d</sup>	12.66 <sup>c</sup>	13.25 <sup>b</sup>	13.34 <sup>a</sup>	0.13
<b>4-Cell wall constituents</b>					
Neutral Detergent Fibre (NDF)	42.75 <sup>b</sup>	42.36 <sup>d</sup>	42.64 <sup>c</sup>	43.40 <sup>a</sup>	0.30
Acid Detergent Fibre (ADF)	28.62 <sup>b</sup>	28.09 <sup>d</sup>	28.48 <sup>c</sup>	29.53 <sup>a</sup>	0.41
Acid Detergent Lignin (ADL)	5.09 <sup>b</sup>	4.99 <sup>d</sup>	5.06 <sup>c</sup>	5.26 <sup>a</sup>	0.08
Hemicellulose*	14.13 <sup>c</sup>	14.27 <sup>a</sup>	14.16 <sup>b</sup>	13.87 <sup>d</sup>	0.12
Cellulose**	23.53 <sup>b</sup>	23.10 <sup>d</sup>	23.42 <sup>c</sup>	24.27 <sup>a</sup>	0.34

<sup>a-d</sup>Means in the same row within each treatment having different superscripts differ significantly (p<0.05), SEM: Standard error of the mean, \*Hemicellulose: NDF-ADF and \*\*Cellulose: ADF-ADL

were also significantly (p<0.05) affected by sprayed Egyptian clover forage with pyridoxine solution with various levels as mentioned above (50, 75 or 100 mg L<sup>-1</sup>). Moreover, the above-mentioned treatments caused significant (p<0.05) increases in Egyptian clover forage yield quantity compared with control plants (Table 2). Furthermore, the foliar treatment of pyridoxine accompanied with cut stages show significant

increases compared with their corresponding controls throughout cut stages of Egyptian clover forage yield.

**Changes in chemical attributes:** Table 3 showed that treating Egyptian clover with 75 and 100 mg L<sup>-1</sup> pyridoxine significantly decreased DM content compared to untreated plants, while, 50 mg L<sup>-1</sup> significantly increased DM

Table 4: Forage cuts and chemical analysis on energetic and nutritive values and cell wall constituents

Items	Egyptian clover forage cut stages				SEM
	First cut	Second cut	Third cut	Fourth cut	
<b>1-Chemical analysis</b>					
Moisture	10.70 <sup>a</sup>	9.88 <sup>b</sup>	9.35 <sup>c</sup>	8.85 <sup>d</sup>	0.26
Dry Matter (DM)	89.30 <sup>d</sup>	90.12 <sup>c</sup>	90.65 <sup>b</sup>	91.15 <sup>a</sup>	0.26
<b>Chemical analysis on DM basis</b>					
Organic Matter (OM)	88.15 <sup>a</sup>	87.60 <sup>b</sup>	87.21 <sup>d</sup>	87.41 <sup>c</sup>	0.18
Crude Protein (CP)	17.40 <sup>d</sup>	17.85 <sup>b</sup>	19.11 <sup>a</sup>	17.72 <sup>c</sup>	0.15
Crude Fibre (CF)	21.77 <sup>b</sup>	17.57 <sup>d</sup>	20.26 <sup>c</sup>	24.81 <sup>a</sup>	0.45
Ether Extract (EE)	3.32 <sup>a</sup>	2.16 <sup>c</sup>	3.35 <sup>a</sup>	2.81 <sup>b</sup>	0.14
Nitrogen-Free Extract (NFE)	45.66 <sup>b</sup>	50.02 <sup>a</sup>	44.49 <sup>c</sup>	42.07 <sup>d</sup>	0.48
Ash	11.85 <sup>d</sup>	12.40 <sup>c</sup>	12.79 <sup>a</sup>	12.59 <sup>b</sup>	0.18
<b>2-Energetic and nutritive values</b>					
Gross Energy (GE)	4094 <sup>a</sup>	4017 <sup>d</sup>	4082 <sup>b</sup>	4041 <sup>c</sup>	10.83
Digestible Energy (DE)	3111 <sup>a</sup>	3053 <sup>d</sup>	3102 <sup>b</sup>	3071 <sup>c</sup>	8.22
Metabolizable Energy (ME)	2551 <sup>a</sup>	2504 <sup>d</sup>	2544 <sup>b</sup>	2515 <sup>c</sup>	6.73
Net Energy (NE)	1429 <sup>a</sup>	1402 <sup>d</sup>	1425 <sup>b</sup>	1411 <sup>c</sup>	3.79
<b>3-Nutritive values (%)</b>					
Total Digestible Nutrients (TDN)	70.23 <sup>a</sup>	68.91 <sup>d</sup>	70.03 <sup>b</sup>	69.32 <sup>c</sup>	0.19
Digestible Crude Protein (DCP)	12.29 <sup>d</sup>	12.68 <sup>b</sup>	13.74 <sup>a</sup>	12.56 <sup>c</sup>	0.13
<b>4-Cell wall constituents</b>					
Neutral Detergent Fibre (NDF)	43.23 <sup>b</sup>	40.47 <sup>d</sup>	42.23 <sup>c</sup>	45.22 <sup>a</sup>	0.30
Acid Detergent Fibre (ADF)	29.29 <sup>b</sup>	25.46 <sup>d</sup>	27.91 <sup>c</sup>	32.06 <sup>a</sup>	0.41
Acid Detergent Lignin (ADL)	5.21 <sup>b</sup>	4.50 <sup>d</sup>	4.96 <sup>c</sup>	5.73 <sup>a</sup>	0.08
Hemicellulose*	13.94 <sup>c</sup>	15.01 <sup>a</sup>	14.32 <sup>b</sup>	13.16 <sup>d</sup>	0.12
Cellulose**	24.08 <sup>b</sup>	20.96 <sup>d</sup>	22.95 <sup>c</sup>	26.33 <sup>a</sup>	0.34

<sup>a-d</sup>Means in the same row within each treatment having different superscripts differ significantly ( $p < 0.05$ ), SEM: Standard error of the mean, \*Hemicellulose: NDF-ADF and \*\*Cellulose: ADF-ADL

content in comparison with the other treatments. Different treatments of pyridoxine occurred significantly ( $p < 0.05$ ) increasing in their CP content, the highest CP content was obtained from 100 mg L<sup>-1</sup> pyridoxine (18.64%).

Despite, CF contents were significantly ( $p < 0.05$ ) reduced by treating Egyptian clover forage with 50 or 75 mg L<sup>-1</sup> pyridoxine (20.45 and 20.88%) as compared with control and 100 mg L<sup>-1</sup> water (21.04 and 22.04% CF, respectively). Pyridoxine foliar treatment with 100 mg L<sup>-1</sup> caused a significant ( $p < 0.05$ ) increase in EE content of Egyptian clover compared to control and the other two levels. The corresponding values of EE content were (3.03, 2.31, 2.85 and 3.46%) for 0, 50, 75 and 100 mg pyridoxine/L water, respectively. All spraying levels of pyridoxine (50, 75 and 100 mg L<sup>-1</sup> water) significantly ( $p < 0.05$ ) decreased their contents of NFE (46.22, 45.26 and 43.89%) in comparison with the control (46.85% NFE). In addition, treating Egyptian clover with pyridoxine with (50 or 75 mg L<sup>-1</sup> water) significantly increased their content of Ash compared to control, meanwhile, 100 mg pyridoxine was noticed at the same range of Ash content with the control.

Table 4 revealed the changes in chemical attributes of Egyptian clover forage all over different cut stages. Results showed significant ( $p < 0.05$ ) differences between the first, second, third and fourth cut stages. Results show the progressive increases in DM throughout the first, second,

third and fourth cuts (89.30%, 90.12, 90.65 and 91.15% DM), respectively. Organic Matter (OM) content was significantly ( $p < 0.05$ ) decreased throughout the different cut stages of Egyptian clover forage (second, third and fourth cuts) compared to control (first cut). Crude Protein (CP), Ether Extract (EE) and Ash contents showed significantly ( $p < 0.05$ ) increases in the third cut stages compared to the others cut stages. Meanwhile, Nitrogen-Free Extract (NFE) content showed a significant ( $p < 0.05$ ) increase in the second cut in comparison with the others cuts. Also, Crude Fibre (CF) content showed a significant ( $p < 0.05$ ) increase in the fourth cut in comparison with the others cuts.

For the interaction of different levels of pyridoxine treatments and cut stages, Table 5 and 6 show the promotive effect of different concentrations of pyridoxine (50, 75 and 100 mg L<sup>-1</sup>) in DM, OM, CP, EE, CF and NFE as compared with the corresponding controls in the four cuts first, second, third and fourth cuts in most treatments except, Ash content of the first cut, OM and EE of the second and third cuts and DM, OM, CF and NFE of the fourth cut different pyridoxine levels decreased plant contents (Table 5).

**Changes in energetic and nutritive values:** Table 3 revealed the enhancing effect of treating Egyptian clover forage with 100 mg L<sup>-1</sup> water caused significant ( $p < 0.05$ ) increases in Gross Energy (GE), Digestible Energy (DE),

Table 5: Interactions between pyridoxine treatment levels and Egyptian clover forage cut stages (chemical analysis, energetic and nutritive values and cell wall constituents Egyptian clover forage cuts)

Items	Pyridoxine treatment levels mg L <sup>-1</sup> water												SEM				
	First cut			Second cut			Third cut			Fourth cut							
	0	50	75	100	0	50	75	100	0	50	75	100	0	50	75	100	
<b>1-Chemical analysis</b>																	
Moisture	9.71 <sup>f</sup>	7.85 <sup>k</sup>	10.73 <sup>c</sup>	14.51 <sup>a</sup>	10.52 <sup>d</sup>	8.71 <sup>i</sup>	9.56 <sup>g</sup>	10.72 <sup>c</sup>	9.79 <sup>e</sup>	10.45 <sup>d</sup>	11.00 <sup>b</sup>	6.16 <sup>m</sup>	7.54 <sup>l</sup>	9.37 <sup>h</sup>	9.33 <sup>h</sup>	9.16 <sup>i</sup>	
Dry Matter (DM)	90.29 <sup>h</sup>	92.15 <sup>c</sup>	89.27 <sup>k</sup>	85.49 <sup>m</sup>	89.48 <sup>i</sup>	91.29 <sup>d</sup>	90.44 <sup>g</sup>	89.28 <sup>k</sup>	90.21 <sup>i</sup>	89.55 <sup>i</sup>	89.00 <sup>i</sup>	93.84 <sup>a</sup>	92.46 <sup>b</sup>	90.63 <sup>f</sup>	90.67 <sup>f</sup>	90.84 <sup>e</sup>	
<b>Chemical analysis on DM basis</b>																	
Organic Matter (OM)	86.18 <sup>n</sup>	88.02 <sup>e</sup>	87.57 <sup>g</sup>	90.82 <sup>a</sup>	88.73 <sup>c</sup>	86.34 <sup>f</sup>	88.40 <sup>d</sup>	86.92 <sup>i</sup>	87.73 <sup>f</sup>	87.06 <sup>f</sup>	86.58 <sup>k</sup>	87.47 <sup>h</sup>	89.41 <sup>b</sup>	85.81 <sup>n</sup>	87.53 <sup>gh</sup>	86.89 <sup>j</sup>	
Crude Protein (CP)	16.00 <sup>i</sup>	16.60 <sup>f</sup>	18.20 <sup>f</sup>	18.81 <sup>c</sup>	17.42 <sup>h</sup>	17.29 <sup>g</sup>	17.91 <sup>g</sup>	18.79 <sup>c</sup>	18.53 <sup>d</sup>	19.54 <sup>b</sup>	19.75 <sup>a</sup>	18.61 <sup>d</sup>	16.41 <sup>k</sup>	17.89 <sup>g</sup>	18.24 <sup>f</sup>	18.34 <sup>e</sup>	
Crude Fibre (CF)	18.10 <sup>i</sup>	22.67 <sup>f</sup>	22.58 <sup>g</sup>	23.73 <sup>d</sup>	17.15 <sup>n</sup>	16.71 <sup>o</sup>	18.68 <sup>h</sup>	17.74 <sup>m</sup>	21.34 <sup>h</sup>	18.26 <sup>k</sup>	18.56 <sup>j</sup>	22.87 <sup>e</sup>	27.56 <sup>a</sup>	24.16 <sup>b</sup>	23.70 <sup>d</sup>	23.82 <sup>c</sup>	
Ether Extract (EE)	3.96 <sup>a</sup>	1.82 <sup>i</sup>	3.65 <sup>c</sup>	3.86 <sup>b</sup>	3.28 <sup>j</sup>	1.03 <sup>m</sup>	1.10 <sup>l</sup>	3.24 <sup>gh</sup>	3.62 <sup>c</sup>	3.31 <sup>ef</sup>	3.27 <sup>g</sup>	3.19 <sup>h</sup>	1.25 <sup>k</sup>	3.09 <sup>j</sup>	3.36 <sup>e</sup>	3.54 <sup>d</sup>	
Nitrogen-Free Extract (NFE)	48.12 <sup>d</sup>	46.93 <sup>f</sup>	43.14 <sup>k</sup>	44.42 <sup>j</sup>	50.88 <sup>b</sup>	51.31 <sup>a</sup>	50.71 <sup>c</sup>	47.15 <sup>e</sup>	44.24 <sup>f</sup>	45.95 <sup>g</sup>	45.00 <sup>h</sup>	42.80 <sup>i</sup>	44.19 <sup>j</sup>	40.67 <sup>o</sup>	42.23 <sup>m</sup>	41.19 <sup>n</sup>	
Ash	13.82 <sup>b</sup>	11.98 <sup>h</sup>	12.43 <sup>h</sup>	9.18 <sup>n</sup>	11.27 <sup>j</sup>	13.66 <sup>c</sup>	11.60 <sup>k</sup>	13.08 <sup>e</sup>	12.27 <sup>i</sup>	12.94 <sup>f</sup>	13.42 <sup>d</sup>	12.53 <sup>g</sup>	10.59 <sup>m</sup>	14.19 <sup>a</sup>	12.47 <sup>gh</sup>	13.11 <sup>e</sup>	
<b>2-Energetic and nutritive values</b>																	
Gross Energy (GE)	4024 <sup>i</sup>	3997 <sup>i</sup>	4099 <sup>d</sup>	4254 <sup>a</sup>	4116 <sup>b</sup>	3897 <sup>k</sup>	3995 <sup>j</sup>	4059 <sup>h</sup>	4109 <sup>c</sup>	4080 <sup>f</sup>	4061 <sup>gh</sup>	4077 <sup>f</sup>	4022 <sup>i</sup>	3992 <sup>j</sup>	4082 <sup>f</sup>	4067 <sup>g</sup>	
Digestible Energy (DE)	3058 <sup>g</sup>	3038 <sup>h</sup>	3115 <sup>c</sup>	3233 <sup>a</sup>	3128 <sup>b</sup>	2962 <sup>j</sup>	3036 <sup>h</sup>	3085 <sup>f</sup>	3123 <sup>b</sup>	3101 <sup>d</sup>	3086 <sup>ef</sup>	3099 <sup>d</sup>	3057 <sup>g</sup>	3034 <sup>h</sup>	3102 <sup>d</sup>	3091 <sup>e</sup>	
Metabolizable Energy (ME)	2508 <sup>g</sup>	2491 <sup>h</sup>	2554 <sup>c</sup>	2651 <sup>a</sup>	2565 <sup>b</sup>	2429 <sup>j</sup>	2490 <sup>h</sup>	2530 <sup>f</sup>	2561 <sup>b</sup>	2543 <sup>d</sup>	2531 <sup>ef</sup>	2541 <sup>d</sup>	2507 <sup>g</sup>	2488 <sup>h</sup>	2544 <sup>d</sup>	2535 <sup>e</sup>	
Net Energy (NE)	1404 <sup>g</sup>	1395 <sup>h</sup>	1430 <sup>c</sup>	1485 <sup>a</sup>	1436 <sup>b</sup>	1360 <sup>j</sup>	1394 <sup>h</sup>	1417 <sup>f</sup>	1434 <sup>b</sup>	1424 <sup>d</sup>	1417 <sup>f</sup>	1423 <sup>d</sup>	1404 <sup>g</sup>	1393 <sup>h</sup>	1425 <sup>d</sup>	1420 <sup>e</sup>	
<b>3-Nutritive values (%)</b>																	
Total Digestible Nutrients (TDN)	69.03 <sup>g</sup>	68.58 <sup>h</sup>	70.32 <sup>c</sup>	72.98 <sup>a</sup>	70.61 <sup>b</sup>	66.86 <sup>j</sup>	68.53 <sup>h</sup>	69.64 <sup>f</sup>	70.50 <sup>b</sup>	70.00 <sup>d</sup>	69.66 <sup>ef</sup>	69.95 <sup>d</sup>	69.01 <sup>g</sup>	68.49 <sup>h</sup>	70.02 <sup>d</sup>	69.77 <sup>e</sup>	
Digestible Crude Protein (DCP)	11.10 <sup>i</sup>	11.61 <sup>i</sup>	12.97 <sup>f</sup>	13.49 <sup>c</sup>	12.31 <sup>h</sup>	12.20 <sup>j</sup>	12.72 <sup>g</sup>	13.47 <sup>c</sup>	13.25 <sup>d</sup>	14.11 <sup>b</sup>	14.29 <sup>a</sup>	13.32 <sup>d</sup>	11.45 <sup>k</sup>	12.71 <sup>g</sup>	13.00 <sup>f</sup>	13.09 <sup>e</sup>	
<b>4-Cell wall constituents</b>																	
Neutral Detergent Fibre (NDF)	40.82 <sup>i</sup>	43.82 <sup>f</sup>	43.76 <sup>g</sup>	44.51 <sup>d</sup>	40.19 <sup>n</sup>	39.90 <sup>o</sup>	41.20 <sup>j</sup>	40.58 <sup>m</sup>	42.94 <sup>h</sup>	40.92 <sup>k</sup>	41.12 <sup>l</sup>	43.95 <sup>c</sup>	47.03 <sup>a</sup>	44.80 <sup>b</sup>	44.49 <sup>d</sup>	44.57 <sup>c</sup>	
Acid Detergent Fiber (ADF)	25.94 <sup>i</sup>	30.11 <sup>f</sup>	30.02 <sup>g</sup>	31.07 <sup>d</sup>	25.07 <sup>n</sup>	24.67 <sup>o</sup>	26.47 <sup>j</sup>	25.61 <sup>m</sup>	28.89 <sup>h</sup>	26.09 <sup>k</sup>	26.36 <sup>l</sup>	30.29 <sup>e</sup>	34.57 <sup>b</sup>	31.47 <sup>b</sup>	31.05 <sup>d</sup>	31.16 <sup>c</sup>	
Acid Detergent Lignin (ADL)	4.59 <sup>i</sup>	5.36 <sup>f</sup>	5.35 <sup>g</sup>	5.54 <sup>d</sup>	4.43 <sup>n</sup>	4.35 <sup>o</sup>	4.68 <sup>j</sup>	4.53 <sup>m</sup>	5.14 <sup>h</sup>	4.62 <sup>k</sup>	4.67 <sup>l</sup>	5.40 <sup>e</sup>	6.19 <sup>b</sup>	5.62 <sup>b</sup>	5.54 <sup>d</sup>	5.56 <sup>c</sup>	
Hemicellulose*	14.88 <sup>d</sup>	13.71 <sup>f</sup>	13.74 <sup>g</sup>	13.44 <sup>e</sup>	15.12 <sup>b</sup>	15.23 <sup>b</sup>	14.73 <sup>g</sup>	14.97 <sup>c</sup>	14.05 <sup>h</sup>	14.83 <sup>e</sup>	14.76 <sup>f</sup>	13.66 <sup>h</sup>	12.46 <sup>i</sup>	13.33 <sup>n</sup>	13.44 <sup>l</sup>	13.41 <sup>m</sup>	
Cellulose**	21.35 <sup>i</sup>	24.75 <sup>f</sup>	24.67 <sup>g</sup>	25.53 <sup>d</sup>	20.64 <sup>n</sup>	20.32 <sup>o</sup>	21.79 <sup>j</sup>	21.08 <sup>m</sup>	23.75 <sup>h</sup>	21.47 <sup>k</sup>	21.69 <sup>l</sup>	24.89 <sup>e</sup>	28.38 <sup>a</sup>	25.85 <sup>b</sup>	25.51 <sup>d</sup>	25.60 <sup>c</sup>	

\*Means in the same row having different superscripts differ significantly (p<0.05), SEM: Standard error of the mean, \*Hemicellulose: NDF-ADF and \*\*Cellulose: ADF-ADL



Metabolizable Energy (ME), Net Energy (NE) and Total Digestible Nutrients (TDN) contents compared with control and the other treatments (50 and 75 mg L<sup>-1</sup>). Meanwhile, spraying Egyptian clover by 50 or 75 mg L<sup>-1</sup> significantly ( $p < 0.05$ ) decreased all energetic values (GE, DE, ME and NE) and nutritive value of TDN as compared to control plants. Meanwhile, Digestible Crude Protein (DCP%) were increased with different levels of pyridoxine treatment to Egyptian clover forage. The most effective concentration was 100 mg L<sup>-1</sup> pyridoxine as it gave 13.34%, in DCP% compared with 12.03% of control, 12.66% and 13.25%, for 50 and 75 mg treating plants.

Table 4 revealed show that values of Gross Energy (GE), Digestible Energy (DE), Metabolizable Energy (ME), Net Energy (NE) and Total Digestible Nutrients (TDN) in Egyptian clover forage decreased significantly in the second, third and fourth cut stages in comparison with the first cut. Meanwhile, digestible crude protein showed an increase in the second, third and fourth cut stages compared to the first cut stage.

Table 5 and 6, the presented data showed the interactive effect of different pyridoxine levels and cut stages. Exogenous treatment of 50, 75 and 100 mg L<sup>-1</sup> pyridoxine significantly increased the various energy and

nutritional values studied in the first and fourth cut, meanwhile, in the second and third cuts, it caused significant decreases in most of the studied parameters as compared with their corresponding values of controls (Table 5).

**Changes in cell wall constituents:** Table 3 showed that exogenous treatment of Egyptian clover with 100 mg L<sup>-1</sup> significantly ( $p < 0.05$ ) increased NDF, ADF, ADL and cellulose contents in comparison with the control plants. Meanwhile, the above-mentioned level (100 mg L<sup>-1</sup>) significantly ( $p < 0.05$ ) decreased the hemicellulose content of Egyptian clover forage.

Data of different cell wall constituents of Egyptian clover as affected by different cut stages are presented in Table 4. The obtained results stated that Neutral Detergent Fibre (NDF), Acid Detergent Fibre (ADF), Acid Detergent Lignin (ADL) and cellulose showed significant ( $p < 0.05$ ) increases in fourth cut stages over the other cut stages of Egyptian clover forage. On the other hand, hemicellulose content showed a significant ( $p < 0.05$ ) decrease in the fourth cut stages compared to the other cut stages.

Table 5 and 6 showed that there were significantly ( $p < 0.05$ ) interactions between pyridoxine levels and berseem

Table 6: ANOVA for chemical analysis on energetic and nutritive values and cell wall constituents

Items	Main effects		
	Pyridoxine treatment levels	Egyptian clover cuts	Interaction
<b>1-Chemical analysis</b>			
Moisture	*	*	*
Dry Matter (DM)	*	*	*
<b>Chemical analysis on DM basis</b>			
Organic Matter (OM)	*	*	*
Crude Protein (CP)	*	*	*
Crude Fibre (CF)	*	*	*
Ether Extract (EE)	*	*	*
Nitrogen-Free Extract (NFE)	*	*	*
Ash	*	*	*
<b>2-Energetic and nutritive values</b>			
Gross Energy (GE)	*	*	*
Digestible Energy (DE)	*	*	*
Metabolizable Energy (ME)	*	*	*
Net Energy (NE)	*	*	*
<b>3-Nutritive values (%)</b>			
Total Digestible Nutrients (TDN)	*	*	*
Digestible Crude Protein (DCP)	*	*	*
<b>4-Cell wall constituents</b>			
Neutral Detergent Fiber (NDF)	*	*	*
Acid Detergent Fiber (ADF)	*	*	*
Acid Detergent Lignin (ADL)	*	*	*
Hemicellulose*	*	*	*
Cellulose**	*	*	*

\*Significant ( $p < 0.05$ ), \*Hemicellulose: NDF-ADF and \*\*Cellulose: ADF-ADL

Table 7: Main consequences of remedies on photosynthetic pigments ( $\mu\text{g g}^{-1}$  fresh wt.) of Egyptian clover plant under sandy soil conditions

Items	Pyridoxine treatment levels $\text{mg L}^{-1}$ water					Egyptian clover cut stages				
	0	50	75	100	SEM	First cut	Second cut	Third cut	Fourth cut	SEM
Total carbohydrates (%)	15.48 <sup>c</sup>	16.20 <sup>b</sup>	16.64 <sup>a</sup>	16.75 <sup>a</sup>	0.13	15.52 <sup>c</sup>	16.09 <sup>b</sup>	17.32 <sup>a</sup>	16.15 <sup>b</sup>	0.13
Total soluble sugars (%)	3.89 <sup>d</sup>	4.34 <sup>c</sup>	5.06 <sup>b</sup>	4.59 <sup>b</sup>	0.09	3.93 <sup>d</sup>	5.03 <sup>a</sup>	4.68 <sup>b</sup>	4.22 <sup>c</sup>	0.09
Polysaccharides (%)	11.59 <sup>c</sup>	11.86 <sup>b</sup>	11.58 <sup>c</sup>	12.16 <sup>a</sup>	0.10	11.59 <sup>c</sup>	11.06 <sup>d</sup>	12.64 <sup>a</sup>	11.93 <sup>b</sup>	0.10
Chlorophyll a	1077 <sup>c</sup>	1165 <sup>b</sup>	1198 <sup>a</sup>	1162 <sup>b</sup>	20.09	962 <sup>d</sup>	1249 <sup>b</sup>	1281 <sup>a</sup>	1109 <sup>c</sup>	20.09
Chlorophyll b	625 <sup>c</sup>	639 <sup>b</sup>	651 <sup>a</sup>	653 <sup>a</sup>	3.12	634 <sup>c</sup>	654 <sup>b</sup>	661 <sup>a</sup>	619 <sup>d</sup>	3.12
Carotenoids	304 <sup>c</sup>	312 <sup>b</sup>	318 <sup>a</sup>	318 <sup>a</sup>	1.54	310 <sup>c</sup>	319 <sup>b</sup>	323 <sup>a</sup>	301 <sup>d</sup>	1.54
Total pigments	2006 <sup>d</sup>	2116 <sup>c</sup>	2167 <sup>a</sup>	2133 <sup>b</sup>	23.43	1906 <sup>d</sup>	2222 <sup>b</sup>	2265 <sup>a</sup>	2029 <sup>c</sup>	23.43
Phenolic compounds	77.39 <sup>d</sup>	84.29 <sup>c</sup>	92.79 <sup>a</sup>	85.34 <sup>b</sup>	2.03	71.40 <sup>d</sup>	75.73 <sup>c</sup>	88.88 <sup>b</sup>	103.81 <sup>a</sup>	2.03
Flavonoids	33.85 <sup>d</sup>	36.86 <sup>c</sup>	40.59 <sup>a</sup>	37.32 <sup>b</sup>	0.89	31.22 <sup>d</sup>	33.12 <sup>c</sup>	38.87 <sup>b</sup>	45.40 <sup>a</sup>	0.89

<sup>a-d</sup>Means in the same row within each treatment having different superscripts differ significantly ( $p < 0.05$ ) and SEM: Standard error of the mean

Table 8: Interactions between pyridoxine treatment levels and Egyptian clover cut stages on photosynthetic pigments ( $\mu\text{g g}^{-1}$  fresh wt.) of the Egyptian clover plant under sandy soil conditions

Items	Egyptian clover cuts												
	First cut			Second cut			Third cut			Fourth cut			
	0	50	75	100	0	50	75	100	0	50	75	100	
Total carbohydrates (%)	14.54 <sup>f</sup>	15.30 <sup>e</sup>	16.25 <sup>c</sup>	16.08 <sup>c</sup>	15.78 <sup>d</sup>	16.20 <sup>c</sup>	16.72 <sup>b</sup>	17.50 <sup>a</sup>	17.46 <sup>a</sup>	15.17 <sup>e</sup>	16.21 <sup>c</sup>	16.54 <sup>b</sup>	16.66 <sup>b</sup>
Total soluble sugars (%)	3.45 <sup>f</sup>	3.52 <sup>f</sup>	4.60 <sup>d</sup>	4.16 <sup>c</sup>	4.27 <sup>e</sup>	4.83 <sup>c</sup>	5.78 <sup>b</sup>	5.24 <sup>b</sup>	4.65 <sup>cd</sup>	3.63 <sup>f</sup>	4.32 <sup>e</sup>	4.65 <sup>cd</sup>	4.29 <sup>e</sup>
Polysaccharides (%)	11.00 <sup>f</sup>	11.78 <sup>cd</sup>	11.65 <sup>cde</sup>	11.92 <sup>c</sup>	11.32 <sup>e</sup>	10.95 <sup>f</sup>	10.42 <sup>g</sup>	12.82 <sup>a</sup>	12.39 <sup>b</sup>	11.54 <sup>de</sup>	11.89 <sup>cd</sup>	11.89 <sup>cd</sup>	12.37 <sup>b</sup>
Chlorophyll a	927 <sup>h</sup>	955 <sup>gh</sup>	993 <sup>f</sup>	972 <sup>ig</sup>	1150 <sup>de</sup>	1273 <sup>c</sup>	1308 <sup>ab</sup>	1263 <sup>c</sup>	1277 <sup>bc</sup>	974 <sup>ig</sup>	1155 <sup>de</sup>	1173 <sup>d</sup>	1134 <sup>e</sup>
Chlorophyll b	612 <sup>k</sup>	624 <sup>j</sup>	637 <sup>g</sup>	665 <sup>bc</sup>	638 <sup>g</sup>	651 <sup>e</sup>	668 <sup>b</sup>	643 <sup>f</sup>	667 <sup>b</sup>	606 <sup>f</sup>	619 <sup>g</sup>	629 <sup>h</sup>	620 <sup>i</sup>
Carotenoids	299 <sup>h</sup>	304 <sup>g</sup>	311 <sup>e</sup>	324 <sup>bc</sup>	311 <sup>e</sup>	317 <sup>d</sup>	326 <sup>ab</sup>	313 <sup>e</sup>	325 <sup>b</sup>	295 <sup>i</sup>	307 <sup>g</sup>	302 <sup>g</sup>	302 <sup>g</sup>
Total pigments	1838 <sup>h</sup>	1883 <sup>g</sup>	1941 <sup>f</sup>	1961 <sup>f</sup>	2099 <sup>d</sup>	2241 <sup>bc</sup>	2302 <sup>a</sup>	2262 <sup>b</sup>	2317 <sup>a</sup>	1875 <sup>g</sup>	2076 <sup>de</sup>	2109 <sup>d</sup>	2056 <sup>e</sup>
Phenolic compounds	63.15 <sup>m</sup>	70.39 <sup>k</sup>	78.77 <sup>h</sup>	73.27 <sup>j</sup>	69.08 <sup>l</sup>	73.90 <sup>i</sup>	83.29 <sup>g</sup>	76.65 <sup>i</sup>	83.44 <sup>g</sup>	94.14 <sup>e</sup>	102.48 <sup>c</sup>	110.62 <sup>a</sup>	107.98 <sup>b</sup>
Flavonoids	27.62 <sup>m</sup>	30.78 <sup>k</sup>	34.45 <sup>h</sup>	32.04 <sup>j</sup>	30.21 <sup>i</sup>	32.32 <sup>i</sup>	36.48 <sup>g</sup>	32.52 <sup>j</sup>	36.49 <sup>g</sup>	41.17 <sup>e</sup>	44.82 <sup>c</sup>	48.38 <sup>b</sup>	47.22 <sup>b</sup>

<sup>a-m</sup>Means in the same row having different superscripts differ significantly ( $p < 0.05$ ) and SEM: Standard error of the mean

Table 9: ANOVA for photosynthetic pigments ( $\mu\text{g g}^{-1}$  fresh wt.) of Egyptian clover plant under sandy soil conditions

Items	Pyridoxine treatment levels			Egyptian clover cuts			Interaction
	*	*	*	*	*	*	
Total carbohydrates (%)	*	*	*	*	*	*	*
Total soluble sugars (%)	*	*	*	*	*	*	*
Polysaccharides (%)	*	*	*	*	*	*	*
Chlorophyll a	*	*	*	*	*	*	*
Chlorophyll b	*	*	*	*	*	*	*
Carotenoids	*	*	*	*	*	*	*
Total pigments	*	*	*	*	*	*	*
Phenolic compounds	*	*	*	*	*	*	*
Flavonoids	*	*	*	*	*	*	*

\*Significant ( $p < 0.05$ )

green forage cut stages on all parameters of chemical analysis, energetic and nutritive values and cell wall constituents.

Table 5 and 6 showed that different concentrations of pyridoxine treatment of Egyptian clover caused significant increases in different cell wall constituents in the first, second and third cuts, meanwhile, decreased them in the fourth cut as compared with untreated control plants throughout the cut stages.

### **Changes in some biochemical and physiological attributes**

**Changes in photosynthetic pigments:** The role of foliar treatment of pyridoxine with different concentrations (50, 75 and 100 mg L<sup>-1</sup>) on various photosynthetic pigments constituents of Egyptian clover forage are tabulated in Table 7. Foliar treatments of various levels of pyridoxine caused significant ( $p < 0.05$ ) increases in chlorophyll a and b, carotenoids and total pigments) throughout first, second, third and fourth cuts. The increases in different photosynthetic pigments were concurrent within pyridoxine concentrations increases. 75 mg L<sup>-1</sup> followed by 100 mg L<sup>-1</sup> gave the highest increases in the studied pigments compared with other treatments in the studied cut stages (Table 7).

Regarding cut stages, the studied photosynthetic pigments constituents (chlorophyll a and b, carotenoids and total pigments) showed gradual and significant increases throughout the studied cut stages from the first cut to the second cut to the third cut then decreased in the fourth cut but still more than first cut.

The interactive effect of different pyridoxine treatments and the four tested cut stages showed the promotive effects of pyridoxine treatments throughout the four cut stages (Table 8) as they caused significant increases in chlorophyll a and b, carotenoids and total pigments).

**Changes in carbohydrates constituents:** The effect of various treatments of pyridoxine treatment on Egyptian clover is shown in Table 7. The obtained results showed the promotive effects of the used concentrations (50, 75 and 100 mg L<sup>-1</sup>) on improving total carbohydrates (%), Total Soluble Sugars (TSS%) and polysaccharides (%) of Egyptian clover cultivated in sandy soil. These increases were significantly and gradually in general concurrent with increasing pyridoxine concentrations compared to the control (untreated plants).

The changes in carbohydrate components throughout the four tested cuts of the Egyptian clover forage plant are tabulated in Table 7. Total carbohydrates (%) and TSS (%)

were increased significantly in the second, third and fourth cuts as compared with those of the first cut. While polysaccharides content in the first cut was reduced significantly in the second cut than increased in the third and fourth cuts over the first cut (Table 7).

Concerning the interactive effects of exogenous treatments of pyridoxine different levels and cut stages the results in Table 8 show that different pyridoxine treatments (50, 75 and 100 mg L<sup>-1</sup>) increased significantly various carbohydrates components of Egyptian clover forage throughout the different cut stages as compared with untreated controls in all the tested cut stages.

**Changes in phenolic and flavonoids contents:** Also, the tabulated results obtained in Table 7 stated that different pyridoxine levels used as foliar treatments (50, 75 and 100 mg L<sup>-1</sup>) on Egyptian clover plants cultivated in sandy soil caused significant increases in both total phenol and flavonoids contents. Increasing in phenolic and flavonoid are gradually in all cut stages.

Table 9 showed the significant ( $p < 0.05$ ) increases in phenolic and flavonoids contents of Egyptian clover as affected by the interactions between pyridoxine treatment levels and the four cut stages.

## **DISCUSSION**

The obtained results of the promotive effect of pyridoxine treatments on forage yield (ton/feddan) Table 1. These promotive effects of pyridoxine are in harmony with those obtained by Zamanipour<sup>15</sup> on tomato, Boghdady<sup>31</sup> on Egyptian lupine and Nassar *et al.*<sup>32</sup> on sesame plant. Barakat<sup>17</sup> found that application of pyridoxine on wheat plants enhanced cell division, increased the root growth and nutrient uptake enhanced efficiency of photosynthetic surface and increased dry matter production. The efficiency of pyridoxine on the growth, yield and quality of berseem was similar to those results investigated by Nassar *et al.*<sup>32</sup> and Younis *et al.*<sup>33</sup>. The treated plants were characterized by the longer main stem which developed more primary leaves number of high specific weight. The positive effect of vitamin B<sub>6</sub> (pyridoxine) was confirmed on the plant's development. In addition, vitamin B could act as an antioxidant in improving plant production<sup>34</sup>. It was confirmed that pyridoxine treatments improve the growth of the root system<sup>35</sup> which causes increases in different nutrient absorption and improved economic productivity<sup>36</sup>.

The promotive effect of pyridoxine on the Egyptian clover forage photosynthetic pigments components are presented

in Table 7. These effects might be caused by the increased activities of various enzymes associated with the biosynthesis of these pigments or the preservation of chromoproteins<sup>37</sup>. Foliar spraying of lupine plants with pyridoxine vitamin with different concentrations improved all fractions of photosynthetic pigments, especially in plants subjected to salt stress. Our obtained results of pyridoxine are in good harmony with those obtained by Hamada and Khulaef<sup>38</sup>, they concluded that treatment of bean plants with 100 ppm pyridoxine stimulated biosynthesis of photosynthetic pigments fractions and net photosynthetic rate. Moreover, Soltani *et al.*<sup>39</sup> confirmed the increased contents of photosynthetic pigments of *Calendula officinalis* L. by treatments of vitamins, they referred these increases to the role of vitamin B as co-enzymes in the enzymatic reactions in carbohydrates, fats and protein metabolism and used in respiration and photosynthesis. Also, Nassar *et al.*<sup>32</sup> on sesame plant. Moreover, the increases in different photosynthetic pigments components were reflected in increasing different physiological parameters as carbohydrates constituents of Egyptian clover plants. These increases in carbohydrate constituents could have resulted from the key effect of vitamins on chlorophyll biosynthesis which in turn increased the biosynthesis of carbohydrates and phenolic as well as flavonoids contents. These results are in agreement with those noticed by Rady *et al.*<sup>40</sup>, El-Metwally and Sadak<sup>41</sup> and Younis *et al.*<sup>33</sup> on different plant species using vitamins treatments. In addition, An increase in total phenols and flavonoids (Table 7) could reduce or inhibit IAA oxidase enzyme activity, thereby increasing IAA levels leading to improved Egyptian clover growth and yield<sup>42</sup>.

Regarding the effect of forage yield throughout the four cuts, the obtained results are in harmony with those obtained earlier by Juskiw<sup>43</sup>, Gaballah<sup>44</sup>, Patel and Rajagopal<sup>45</sup>, Yucel *et al.*<sup>46</sup> and Shahrajabian<sup>47</sup>. In this content, Soleymani and Shahrajabian<sup>48</sup> and Thalooh *et al.*<sup>49</sup> reported that the highest fresh yield was recorded for the 2nd cut followed by the 1st cut then the 3rd one and dry weight (g/m<sup>2</sup>) increased from the 1st to the 2nd cut up to 3rd cut. Moreover, El-Karamany *et al.*<sup>50</sup> noted that the production of dry forage increased from 1st-2nd and 3rd harvest after treatment with bioorganic+mineral fertilizers (1.1432.026 and 3.093 ton/fd.) followed by the mineral fertilization treatments that produced 0.934, 1.838 and 2.746 ton/fad., for the successive three cuts of Egyptian berseem clover. Moreover, this is in agreement with the results obtained by El Karamany *et al.*<sup>50</sup>, Omer *et al.*<sup>51</sup>, Abdel-Magid *et al.*<sup>52</sup> and Salama<sup>53</sup>, who noticed that clover hay on a dry matter basis (in average) contained 92.00, 87.17,

13.40, 26.03, 4.03, 43.71, 43.20, 30.06, 5.54%, 4153 and 2661 kca kg<sup>-1</sup> DM of DM, OM, CP, CF, EE, NFE, Ash, NDF, ADF and ADL, Gross Energy (GE) and Digestible Energy (DE), respectively.

From the results of our study, we found that all pyridoxine levels realized significant increases in Egyptian clover yield quantity as well as their crude protein content while crude fibre content was significantly decreased by spraying Egyptian clover by 50 or 75 mg L<sup>-1</sup> compared with control and 100 mg L<sup>-1</sup>. Furthermore, all levels of pyridoxine significantly decreased the contents of nitrogen-free extract. Therefore, we recommend from our study, the cultivation of Egyptian clover and treatment with peroxide in sandy soils. So we can be concluded that pyridoxine can be safely used without adversely affecting the cultivated plants and this has led to an increase in feed yield and an improvement in nutritional value.

## CONCLUSION

This study discovers the promotive role of exogenous pyridoxine as natural vitamin on plants especially on Egyptian clover that can be beneficial for increasing yield and yield component as well as improving its nutritive value. Pyridoxine as a natural and safely plant component affect plant growth and productivity without adversely affecting the cultivated plants, through increasing photosynthetic pigments, IAA contents as well as, increasing content of DM, OM, CP, CF, EE, NFE, Ash, NDF, ADF and ADL, Gross Energy (GE) and Digestible Energy (DE). So, this study will help the researcher to uncover the critical areas of pyridoxine as a natural to improve plant growth and productivity under sandy conditions.

## SIGNIFICANCE STATEMENT

This study discovers the possible promotive role of vitamins especially Pyridoxine in increasing growth and foraging yield quantity of Egyptian clovers all over the four cuts this study will help the researcher to uncover the critical areas of pyridoxine as a natural to improve plant growth and productivity under sandy conditions.

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