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EFFECT OF SEASONAL VARIATION ON SECONDARY METABOLITES AND NUTRITIVE VALUE OF *Crotalaria aegyptiaca* Benth.

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This study aimed to investigate the effect of seasonal variation on secondary metabolites and the nutritive value of *Crotalaria aegyptiaca* Benth., family Leguminosae, collected from Wadi Hagul at 15km from the beginning of the Wadi during winter and summer seasons.

Crotalaria aegyptiaca grows under high degree of aridity. Na^+ ions form the major cations while Cl^- ions form the major anions in the soil supporting the plant at Wadi Hagul habitat. Samples taken from the plant during summer attained the highest of succulence ratio.

The percentages of total available carbohydrates, total ash, total crude fibres, total protein and total fat content reached their maximum values in summer, while reached their minimum values during winter. The dry matter content of *C. aegyptiaca* reached its maximum values during summer season.

Ca^{++} and Mg^{++} ions were accumulated in high amounts in summer to counteract the harmful effect of Na^+ and Cl^- ions.

The plant samples contained fourteen amino acids in winter with various concentrations, glutamic acid was the highest separated one, while plant samples during summer, season contained seventeen separated amino acids, arginine forms the highest one.

Concerning the active constituents of the whole plant (during summer and winter) and seeds, it was found that the percentages of total flavonoids and total akaloids were higher in seeds than in the whole plant and also were higher during winter than summer (for the whole plant). While the total percentages of saponin content were higher in plant seeds than that of whole plant, and were higher during summer season than winter samples for whole plant.

Meanwhile, the percentages of total tannins were higher in whole plant during summer season.

The nutritive value of *C. aegyptiaca* was determined by two methods and indicated high nutritive value during its vegetative stages with no harmful effect to the animals during overgrazing. However, at the end of seeding stages, the total alkaloids, total flavonoids and total saponin content reached the highest values, so it must be prevented from animal feeding especially during these stages as it would cause animal death.

Keywords: *Crotalaria aegyptiaca,* nutritive value, flavonoids, alkaloids, saponins, tannins.

Studies on wild edible plants and their nutrient composition while are few, they are of crucial importance as they provide necessary information regarding the nutritional status for the local plants and for the human population. Many shrubs and trees in arid regions are of structural and economic importance. They play an important role in soil protection and stabilization against soil erosion by wind or water; provide a source of forage for animals and fuel for local inhabitants in addition to the medicinal and potential industrial values (Thalem, 1979).

Nutritional deficiencies of plants in different environments are generally recognized as a result of the inability of the soils of the affected areas to make the nutrients available to the plants (Underwood, 1971). Desert animals depend mainly on the natural vegetation as the principal feed resource. The green biomass produced from the rangeland vegetation in this region, particularly in dry seasons, may not sustain the nutritional requirements for grazing animals, which could be reflected upon low productivity. Feed resources in such regions could be increased by different approaches, one of them include processing of less palatable and non palatable halophytic shrubs which are naturally and intensively grown to improve their quality (Fahmy, 2001).

Over 80% of the organic compounds found in the natural world are of plant origin. The natural plants usually produce different kinds of natural secondary metabolites during their metabolism, where the nature and amounts of these compounds vary according to the prevailed environmental conditions secondary metabolites are mainly terpenoids, nitrogen – containing compounds and phenolics. Some of these compounds have an essential role in growth and development but the majority of them are involved in chemical defence systems, which protect plants from herbivores, microbial infection and protect them from the consequences of environmental stress (Crawley, 1997). Secondary metabolites represent the accumulation of end - products from relatively lengthy pathways of enzyme-catalysed steps in biosynthesis. These substances assist the growing plant in its struggle for survival in a hostile environment, surrounded by herbivores animals of different types, subject to invasion by parasitic micro-organisms and surrounded by competing plant species.

Leguminosae family (pea family) considered to be one of the most important family for food plants used in many developing countries as stable diet. The presence of bacterial nodules (containing *Rhizobium*) makes many species of importance to soil improvement as well as for fodder production (Ghazanfar, 1994).

Heneidy (2000) reported that family Leguminosae had the highest contribution (33%) among all the families and includes the most important browse rangeland species in the study area (Bisha, Asir region, South Western of Saudi Arabia). He stated that, about 78% of the palatable species belonged to the family Leguminosae.

Crotalaria aegyptiaca, Natash in arabic, belonging to family Leguminosae is a green plant grazed by camels and gazelles, poisonous to sheep and goats if grazed in large quantities. Thus the present study aimed to investigate the effect of seasonal variation on nutritive value and secondary metabolites of *Crotalaria aegyptiaca*.

MATERIALS AND METHODS

This study was carried out at two seasons (winter and summer) on the whole plant at Wadi Hagul in the eastern desert of Egypt at 15Km from the beginning of the Wadi during the growth season of 2002. Seeds of *C. aegyptiaca* were collected during summer season.

Environmental conditions

Climatic factors

The mean values of climatic particulars for Cairo-Suez road were obtained from the Meteorological Department of Egypt during the period of investigation.

Edaphic factors

The soil profiles supporting *C. aegyptiaca* were sampled close to the naturally growing plants. Soil samples were taken from depths of 0 – 20 cm and of 20 - 40 cm, then samples were air dried, sieved through 2mm meshes and kept for further analysis.

a- Soil physical and chemical properties

Soil texture was determined by the sieve method (Jackson, 1967), while soil chemical properties including the hydrogen ion concentration of the soil (pH) was measured using electric pH meter and electrical conductivity (E.C.) was measured by electrical conductivity meter (Rowell, 1994).

b- Anions

Sulphate ions were detemrined by precipitation as barium sulphate (Jackson, 1967) and chloride was determined according to the method described by Brower and Zar (1984).

c- Cations

Sodium and potassium ions were measured using flame photometer according to Rowell (1994), while calcium and magnesium were measured by titration against EDTA (Brower and Zar 1984).

Determination of the nutritive value

The nutritive value of *C. aegyptiaca* was determined during summer and winter using two methods. The first method was made to estimate the total carbohydrates in the whole plant (Chaplin and Kennedy 1994), total ash content and crude fibres (Askar and Treptow 1993), total protein content (James, 1995) and total fat (lipid) percentages (British pharmacopeia, 1998). On the other hand, the second method utilized the nylon bag technique as follows:

Six ruminaly cannulated male Barki sheep of average body weight of 56.5 $\pm$ 1.44 kg were used to determine the disappearance of dry matter (DM), organic matter (OM) and nitrogen contents in the rumen (N). Sheep were offered berseem hay *ad lib* and had free acess to fresh water. Animals were kept in individual cages at Maryot Research Station belong to Desert Research Center.

After 15 days of adaptation in the individual cages, disappearance of DM, OM and N content in *C. aegyptiaca* were determined in summer and winter using the nylon bag technique (Meherz and Ørskov, 1977) in three sheep selected randomly from the station sheep flock. Approximately 3 grams of air-dry whole plant were grounded to 2mm and kept in each nylon bag which measueed 7 x 15 cm and made from polyester cloth (Swiss Nylon Monofilament, Switzerland) with pore size of 50 µm. Two bags were inserted into their rumen of each animal through the cannula, then the bags were withdrawn at incubation time of 72 hours. After withdrawn from the rumen, the bags were washed under running tap water until the water became clear and then squeezed gently. Microorganisms attached to the residual samples were eliminated by freezing-rethawing techniques as described by Kamel *et al.* (1995). The washing loss of DM, OM and N were determined by washing the bags in running water for 30 min. Bags were dried in forced air oven for 24 h at 60°C, cooled in a disicator and weighted. Redrying was made for sub-sample at 105°C to adjust the dry matter content DM, OM and N were estimated according to the method of A.O.A.C. (1990).

Degree of succulence

The degree of succulence of the whole plant was calculated according to Dehan and Tal (1978).

Elemental analysis of the plant

The acid digest was analysed for Na^{+}, K^{+}, Ca^{++}, Mg^{++} and P^{+++}. The contents of Na^{+}, K^{+}, Ca^{++} and Mg^{++} were determined using Atomic Absorbtion Spectrometer SP 1900. Phosphorus content in the digested samples was determined colorimetrically by the molybdic acid method as described by Humphries (1956). Chloride content of the ashed powdered sample after being extracted with 0.1N nitric acid was determined using the $AgNO_3$ method (Jackson and Thomas, 1960). Sulphates were determined gravimetrically using barium chloride according to A.O.A.C. (1975).

Organic matter content in the whole plant samples

Organic matter content was determined by the method described by Jackson (1958).

Determination of protein amino acids (Quantitively)

Analysis of amino acids was carried out using amino acid instrument according to the method described by Steven *et al.* (1989). LKB alpha plus high performance amino acid analyzer LKB biochrom, LTD England, was used for this purpose. Retention times and areas were determined using Hewlett Packard 3390 recording integrator. A special designed computer programme calculated the concentration of each amino acid as μg/100g d.wt.

Active constituents

Preliminary phytochemical screening

It included test of flavonoids (Wall *et al.* 1954), alkaloids (Woo *et al.* 1977) and for saponins and tannins (Balbaa, 1986).

Estimation of total active constituents

The total flavonoid (as kaempferol) of *C. aegyptiaca* at the two studied seasons were determined spectrophotomitrically according to Karawya and Aboutable (1982). The total alkaloids were estimated using two methods, the acid base titration and the gravimetric methods (Balbaa, 1986; Woo *et al.* 1977). Saponin content was determined according to Balbaa (1986). Whereas total tannins were estimated according to Makkar and Goodchild (1996).

RESULTS AND DISCUSSION

Plant chemical analysis

Climatic factors

The climatic particulars are represented in table (1), to indicate the conditions prevailing in the studied area where *Crotalaria aegyptiaca* naturally grows. Climatic data showed that the minimum temperature varied from 10.7°C in January to 24.3°C in July and August. The monthly temperature was 15.1°C in January and 29.5°C in July (Table 1). The maximum temperature varied from 19.8°C in January to 36.0°C in July. Relative humidity ranged from 45% in May to 60% in December. The minimum value of evaporation was 5.6 mm/day in December, while the maximum amount was 11.9 mm/day in June. Data also indicated that the total annual rainfall was 20.6 mm. The dry period covered four months from June to September.

TABLE (1). Mean values of climatic conditions taken for Cairo-Suez road throughout of study.

Months	Temperature °C			R.H.%	Evap. (mm/day)	R.F. (mm)	W.V. (knot/hr)
	Mean Max.	Mean Min.	Monthly Mean				
January	19.8	10.7	15.1	58	5.8	3.1	6.9
February	21.4	11.5	16.2	56	6.5	3.6	7.8
March	23.8	13.2	18.2	53	7.7	5.0	8.3
April	28.5	16.6	22.2	46	10.1	1.5	9.5
May	32.3	19.8	25.6	45	11.3	0.2	9.5
June	35.0	22.7	28.3	48	11.9	0.0	9.7
July	36.0	24.3	29.5	52	11.5	0.0	8.8
August	35.6	24.3	29.4	54	10.8	0.0	8.8
September	33.4	23.1	27.7	56	9.9	0.0	9.4
October	30.0	20.3	24.6	58	8.7	0.3	8.6
November	24.9	15.6	20.0	59	6.7	2.2	7.0
December	20.9	12.0	16.2	60	5.6	4.7	6.3

Max. = Maximum Min. = Minimum
R.H. = Relative humidity Evap. = Evaporation power
R.F. = Rainfall
W.V. = Wind Velocity (Knot = 1.85 km/hour).

It is indicated that *C. aegyptiaca* grows and flourishes well under conditions of moderate temperature; especially during its vegetative growth seasons. The study area was characterized by high degree of aridity as shown by the low values of rainfall, high temperature, especially during long rainless seasons, moderate relative humidity and moderate rate of evaporation (Table 1).

Soil physical and chemical analysis

The physical properties of the soil supporting *C. aegyptiaca* were outlined in table (2), while its chemical analysis were outlined in table (3).

TABLE (2). Physical properties of the soil supporting *Crotalaria aegyptiaca* at Wadi Hagul.

Depth (cm)	Mechanical analysis %				Texture class	Saturation point %	Field capacity %
	Coarse sand	Fine sand	Silt	Clay			
0-20	34.6	60.1	4.0	1.3	Sand	20.1	4.3
20-40	70.5	26.9	0.03	2.6	Coarse sand	16.5	3.7

TABLE (3). Chemical analysis of the soil supporting *Crotalaria aegyptiaca* at Wadi Hagul.

Depth (cm)	EC $mScm^{-1}$	pH	Soluble cations (meq/100g dry soil)				Soluble anions (meq/100g dry soil)		
			Na^+	K^+	Ca^{++}	Mg^{++}	Co_3^-	Cl^-	So_4^{--}
0-20	1.6	7.6	70.2	6.8	46.2	23.6	Traces	24.1	15.1
20-40	2.1	8.0	43.1	2.9	43.1	20.1	Traces	12.5	2.7

It is obvious from table (3) that Na^+ form the major cations while chloride ions form the major anions in soil supporting *C. aegyptiaca* at Wadi Hagul habitat.

TABLE (4). Anions and cations content of *Crotalaria aegyptiaca* . during summer and winter seasons.

Seasons	Cations (meq/100g dry weight)					Anions (meq/100gm dry weight)	
	Na^+	K^+	Ca^{++}	Mg^{++}	P^{+++}	Cl^-	So_4^{--}
Winter	10.0	3.4	93.2	16.1	5.7	40.0	2.6
Summer	5.0	10.2	174	22.0	12.1	46.0	2.9

Mineral composition

From the obtained data, it was observed that the total ash percentages tend to increase in the dry season (summer) while declined in the rainy season (winter). This coincided with the rhythm of climatic conditions as well as with seasonal changes in soil moisture content and the level of soluble salts in the soil.

C. aegyptiaca showed the maximum content of Na^+ in winter (10.0 meq/100g dry wt) and the minimum value in summer (5.0 meq/100 g dry wt). Maximum K^+ ion accumulation was achieved by plants during summer (10.2 meq/100 g dry wt), whereas the lowest one was observed in winter (3.4 meq/100 g dry wt). Potassium promotes certain enzyme reactions and acts with sodium to maintain normal pH levels and responsible for the balance between fluids inside and outside the cell (Lust, 1974). Potassium is an important contributor to the osmotic potential of the cells (Pessarakli, 1995) while calcium is an important mineral for the construction of cell walls.

Data presented in table (4) indicated that the highest Ca^{++} content (174.0 meq/100 g dry wt) was attained during summer season while its amount during winter reached 93.2 meq/100 g dry wt. So, the accumulation of calcium was shown in the plants during summer. Pessarakli (1995) reported that calcium pectate is the major structural chemical of the middle lamella of cell wall. Magnesium form the second major cation in *C. aegyptiaca* during summer and winter seasons (Table 4). It ranged between 16.1 meq/100 g dry wt during winter and 22.0 meq/100 g dry wt in summer.

Ca^{++} content occurs in both plant and animal tissue, it is probably involved in the formation and maintenance of body protein and being essential as an enzyme activator (Lust, 1974). It is a cofactor of a number of enzymes, including those involved in the phosphorylation processes (Pessarakli, 1995).

As far as the accumulation of Ca^{++} and Mg^{++} in high amounts in summer to counteract the harmful effect of Na^{+} and Cl^{-} ions. Several workers (Epstein, 1972 and Waisel, 1972) reported on the crucial role played by Ca^{++} and Mg^{++} ions in the response of plants to salts. They suggested that tolerance of plants to high levels of Na^{+} ions was related to the availability of Ca^{++} and Mg^{++} ions. Phosphorus content ranged between a minimum of 5.7 meq/100 g dry wt of *C. aegyptiaca* in winter and a maximum of 12.1 meq/100 g dry wt in summer. Kilmer *et al.* (1960) showed that phosphorus availability and uptake increase with the increase in soil moisture stress. Phosphorus occurs widely in both plant and animal tissue. It takes part in the production of energy. It is necessary for metabolites functions and enzyme formation (Lust, 1974). It plays an important structural role, as the backbone of nucleic acid structure and as a component of membrane phospholipids (Pessarakli, 1995).

Chloride content

Chloride content of *C. aegyptiaca* reached the maximum value of 46.0 meq/100 g dry wt during summer and minimum value of 40.0 meq/100 g dry wt in winter (Table 4).

Generally, Cl^{-} content increased on passing from the rainy season to the end of the dry season. Natural xerophytic plants increased more by univalent ions than by divalent or trivalent ions. Chloride ions have specific and osmotic effects on the protoplasm and have visible manifestation in increasing leaf succulence (El-Monayeri *et al.* 1986).

Sulfate content

Sulfate ions are taken up by plants mostly in smaller amounts than Cl^{-} ions and are used for amino acids and enzymes synthesis and for counteracting the increased Cl^{-} ions accumulation (Strogonov, 1964). It was noticed from table (4) that So_4^{--} content of *C. aegyptiaca* was somewhat low as compared with their Cl^{-} content. The highest amount of So_4^{--} (2.9 meq/100 g dry wt) was attained during summer, while the lowest amount (2.6 meq/100 g dry wt) was observed during winter.

Succulence

Samples taken from the whole plant during summer (Table 5) attained the highest of succulence ratio (3.9) while winter samples attained the lowest values (2.4). Greenway and Munns (1980) concluded that succulence is one of the most common features of many obligate and facultative halophytes as well as some xerophytes. Succulence in plants increased more by univalent ions than by divalent or trivalent ions.

Determination of the Nutritive Value of Crotalaria aegyptiaca

Total available carbohydrates

The total available carbohydrates (TAC) include soluble and convertible sugars. Under moisture stress conditions, insoluble carbohydrates are converted to soluble sugars that lead to lower osmotic potential and consequently the plant water potential. The differences in water potential gradients are responsible for water, organic and inorganic solutes translocations in plant cells (Stocker, 1961).

It is clear from table (5) that the percentages of TAC reached their maximum value of 5.6% in summer months, while minimum value was 4.3% during winter. Carbohydrate content tend to decrease in the rainy season and to increase at the end of the dry season.

Ash conten

Data presented in table (5) indicate that the total ash content of *C. aegyptiaca* reached its maximum value of 12.1% in summer and its minimum value of 5.2% in winter. The rise of ash content during summer may be due to the increase in total ion accumulation as a result of increasing soil moisture stress (El-Monayeri *et al.* 1981). Also, El-Monayeri *et al.* (1986) stated that ash content was higher during the dry period than during the wet period. This coincides with the rhythm of climatic conditions as well as with seasonal changes in soil moisture content. They also stated that total ash content as well as the individual minerals of Na^+, Cl^-, K^+, Mg^{++} and Ca^{++} increased in tissues with the increase of soil moisture stress which coincides with the dry season. The ash content of plant material is a good criterion of the total mineral contents as it includes all ions analyzed in addition to others in the form of oxides.

TABLE (5). Chemical composition of *Crotalaria aegyptiaca* to determine the nutritive value.

Seasons	Succulence	Total available carbohydrates %	Total ash %	Crude fibers %	Total protein %	Total fat %
Winter	2.4	4.3	5.2	20.4	9.38	8.6
Summer	3.9	5.6	12.1	36.5	13.13	9.8

Heneidy (2000) determined ash content in the grazeable parts of certain species belonging to Leguminosae family such as *Acacia ehrenbergiana* (7.5%), *A. tortilis* (9.3%), *Cassia italica* (13.9%), *Indigofera spinosa* (3.9%), *Lygos raetam* (4.9%), *Tephrosia nubica* (5.7%) and *Trigonella foenum-graceum* (12.9%).

Crude fibres

Crude fibres of *C. aegyptiaca* reached its maximum value of 36.5% in summer, while its minimum value of 20.4% was recorded during winter (Table 5). As a general conclusion, the total ash, fat, total available carbohydrates, total nitrogen and protein and crude fiber content were higher in summer than in winter, which may be attributed to the lower moisture content. Crude fibres content showed seasonal fluctuation in winter and summer because of the response of the plant to different stress conditions in both seasons.

Crude fiber in the grazeable parts of some plant species belonging to family Leguminosae were determined (Heneidy, 2000), *Acacia ehrenbergiana* (20.4%), *A. tortilis* (13.1%), *Cassia italica* (32.9%), *Indigofera spinosa* (34.2%), *Lygos raetam* (32.5%), *Tephrosia nubica* (38.0%) and *Trigonella foenum-graceum* (17.1%).

Total protein content

Data presented in table (5) indicate that the percentages of total protein of *C. aegyptiaca* reached its maximum value of 13.13% in summer and its minimum value of 9.38% in winter. In general, the amount of protein decreased during winter months and decreased during summer months. The increase in the soil moisture stress may remarkably increase the assimilation and accumulation of nitrogenous compounds in *C. aegyptiaca*. A behaviour similar to the response of carbohydrates to soil drought conditions.

Bryant *et al.* (1983) concluded that crude protein is commonly viewed as an indicator of the nutritional value of plants as food for ruminants.

Total fat content

The total lipid content of *C. aegyptiaca* reached its maximum value of 9.8% during summer and its minimum value of 8.6% during winter (Table 5). It is obvious from the obtained data that the total lipid percentages are higher in summer season than in winter. This may be due to the increase in the metabolic rate of *C. aegyptiaca* plant during summer, which leads to increase carbohydrate content, which converted to lipid by oxidation reactions. These results were in agreement with those obtained by Stocker (1960).

Nutritive Value of Crotalaria aegyptiaca by the Nylon Bag Technique

Results in table (6) revealed that the disappearance of dry matter (DM) *in vivo* reached its maximum value of 84.7% during winter season while reached 46.4% during summer season. The chemical composition revealed that the disappearance of dry matter, organic matter and crude protein reached their maximum values during winter season.

El-Shaer *et al.* (1984) reported that, the highest values of crude protein, ether extract, nitrogen free extract and nylon bag-dry matter disappearance value were recorded in winter and spring while the lowest values were obtained in autumn. The reverse trend was recorded for dry matter, ash and crude fiber contents.

TABLE (6). Chemical content *in vivo* dry matter disappearance in *Ctrotalaria aegyptiaca* after 72 hours.

Season	In vivo dry matter disappearance	Chemical composition		
		Disappearnce of dry matter	Organic matter	Crude protein
Summer	46.4	73.6	77.8	2.1
Winter	84.7	93.6	97.3	3.5

Protein amino acids

Crotalaria aegyptiaca contained fourteen amino acids in winter sample and seventeen amino acids in summer sample, with different ranges of concentration. It is obvious from table (7) that glutamic acid was the highest separated amino acid (after protein hydrolysis) with concentration of 504.7 μg/100 g dry wt. in winter sample, while the lowest detected amino acid in protein fraction of the plant was tyrosine (136.8 μg/100 g dry wt.). In summer sample, arginine (285.5 μg/100 g dry wt.) was the highest separated amino acid, meanwhile, methionin (54.1 μg/100 g dry wt.) was the lowest detected amino acid in protein fraction. Proline (146.2 μg/100 g dry wt.) was detected only in summer sample.

TABLE (7). Protein amino acids of *Crotalaria aegyptiaca* using Amino Acid Analyzer at different seasons.

Peak No.	RT	Name of amino acids	μg/100 g dry wt.	
			Winter	Summer
1	10.07	Aspartic acid	492.8	153.1
2	13.45	Therionine	289.9	101.3
3	14.79	Serine	284.2	120.6
4	16.25	Glutamic acid	504.7	210.0
5	18.41	Proline	-	146.2
6	22.07	Glycine	250.2	143.2
7	23.05	Alanine	211.7	118.7
8	25.53	Valine	282.3	149.8
9	27.16	Methionin	-	54.0
10	28.83	Isoleucine	258.7	106.3
11	29.88	Leucine	358.3	165.3
12	33.79	Tyrosine	136.8	66.3
13	36.43	Phenylalanine	326.2	74.7
14	44.76	Histidine	221.0	140.5
15	47.47	Lysine	362.2	145.6
17	54.81	Arginine	-	285.5

The responses of plants to environmental stresses are varied and generally involve some alternation in protein synthesis. Quantitative and qualitative changes in the synthesis of proteins have been reported to occur in plants in response to water deficit (Pessarakli, 1995). The physiological significance of proline accumulation may due to its role in osmoregulation, provision of both carbon and nitrogen for post-stress relief or to the removal of ammonia (Barnett and Naylor, 1966). Proline has been suggested to act as a compatible cytoplasmic osmoticum, may enhance salt tolerance and be a symptom of cell damage during salt/water stress. Moreover, proline accumulation is important for cell growth (Zidan and Elewa, 1992). Antunes and Campos (1995) stated that thirty-four amino acids from seeds of 8 *Phaseolus* species (Leguminosae-Phaseoleae) were analysed by two dimensional paper chromatography and ion-exchange chromatography, showed their taxonomic and nutritional significance.

Preliminary phytochemical screening

It appears that *C. aegyptiaca* plant and seeds contained tannins, alkaloids, saponins and flavonoid and phenolics. Ghazanfar (1994) reported the presence of alkaloids and saponins in species of *Astragalus* (Family: Leguminosae). Onwukaeme (1995) reported the presence of tannins, flavonoids and saponin glycosides in *Baphia nitida* (Leguminosae), leaves were used in folk medicine for the treatment of inflamed and infected umbilical cords and as anti-inflammatory agent.

TABLE (8). Percentages of total active constituents of *Crotalaria aegyptiaca* during the period of investigation.

Item	Whole plant sample		Seeds
	Winter	Summer	
Total tannins %	4.16	4.52	1.17
Total flavonoids %	3.65	1.90	3.66
Total saponins %	1.18	1.90	2.33
Total alkaloids %:			
1-Acid base titration method	1.49	0.28	2.39
2- Gravimetric method	1.47	0.25	2.36

Estimation of total flavonoids

The flavonoids of *C.aegyptiaca* were determined spectrophotometrically and calculated as kaempferol. The percentages of total flavonoids were 3.65 and 1.90 for the whole plant during winter and summer, respectively. While the percentage of total flavonoids of plant seeds were 3.66 (Table 8).

Data indicated that the percentages of total flavonoids were higher in plant seeds than the whole plant and were also higher during winter than summer. The results obtained from this study indicate that the activity of enzymes involved in the synthesis of flavonoids beginning in summer and increasing as winter progresses. This quantitative variation of the flavonoids must be derived from induction by environmental factors to which the plant is subjected. Inderjit and Foy, (1999) reported that flavonoids have been further adopted for a number of other uses by the plants and the animals that consume them. In plants, they appear to have diverse functions, including functioning as antioxidants, superoxide radical scavengers, chelators mediating mineral uptake, enzyme inhibitors and regulators, redox cofactors and pigmented color attractants for pollination and seed dispersal mechanisms by insects and animals. They also stated that flavonoids biosynthesis may also be induced by exogenous stimuli, such as changes in light and temperature.

Conditions of environmental stress may restrict plant growth and photosynthetic rate. Such conditions lead to the synthesis of compounds deriving from secondary metabolism, with the most important group being the flavonoids.

Estimation of total alkaloids

Data presented in table (8) indicated that the percentages of total alkaloids of *C. aegyptiaca* were 1.49 and 0.28 using acid base titration method and 1.47 and 0.25 using the gravimetric method in winter and summer, respectively. The total alkaloids of seeds were 2.39% and 2.36%, being higher in winter than in summer.

Alkaloids are the best known of the nitrogen-containing secondary metabolites of plants. They are efficiently used as defensive agents and they may be moved around the plant to those parts needing greater proteciton during growth and development. Alkaloids are physiologically active at very low concentrations while others, such as the tannins, exert their deleterious effects at relatively high concentration. Alkaloids and glycosides comprise two major classes of plant toxins, both highly complex and diverse.

Rizk (1986) stated that the genus *Crotalaria* has proven to be a valuable source of pyrrolizidine alkaloids. Monocrotaline was isolated from *Crotolaria aegyptiaca* and *C. mysorensis*. Roeder *et al.* (1993) isolated six pyrrolizidine alkaloids, including two new compounds named croaegyptine and crotalarine lactone, from *Crotalaria aegyptiaca* collected from the Red Sea Coastal region in Egypt.

Estimation of total saponins

The total saponins of *C. aegyptiaca* was illustrated in table (8). The percentages of total saponins of seeds were 2.33 while for the whole plant, reached from 1.08 and 1.90 for winter and summer seasons. Data indicated that the percentages of total saponins of *C. aegyptiaca* seeds were higher than that of the whole plants, while the percentages of total saponins were higher in summer than in winter.

Saponins are groups of steroids or triterpenes natural products occuring in many plant families. The ecological roles of this group has been defined with regard to certain plant-microbes, plant-plant or plant-insect interactions. A function of certain saponins as protectants from phytopathogen infection and insect predation suggests that they may have fungicidal and insecticidal roles in nature. Differential synthesis and/or accumulation of saponins and their aglycones is observed in different plant tissues and organs depending upon species or genotype, age and environmental conditions (Inderjit and Foy, 1999).

Rastrelli *et al.* (1999) isolated two new triterpene saponins and known aromatic compounds from *Gliricidia sepium* (Leguminosae) leaves.

Estimation of total tannins

Data in table (8) showed that the percentages of total tannins were higher during summer than winter for whole plant samples. The percentage of total tannins of seeds was 1.17, while for the whole plants reached 4.16 and 4.52 during winter and summer samples, respectively.

High concentration of condensed tannin in the vegetation facilitates accumulation of organic matter. From table (6) it was observed that the dry matter content of *C. aegyptiaca* reached the highest value of 26.22% during summer, while the tannin content reached the maximum value of 4.52%. Moreover, during winter, the dry matter content reached the minimum value of 25.6% coincident with low content of tannin (4.16%).

Tannins are water-soluble phenolic compounds, have special properties such as the ability to precipitate alkaloids, gelatin and other proteins. Tannins have molecular weights lying between 500 and 3,000 and can reach 5,000, so tannins contain sufficient phenolic hydroxyl groups to permit the formation of stable cross-links with proteins, and as a result cross-linking enzymes may be inhibited. Because of these protein-binding properties, they are of considerable importance in food processing and fruit ripening (Bianco and Savolainen, 1997).

The family Leguminosae has been reported rich in tanniniferous plants. The woods of some plants are also well-known tanning materials, e.g. *Acacia catechu* and *Castanea sativa*, moreover, the pods may be a rich source for tannins in this family as those of *Caesalpinia coriaria* and *Acacia arabica*. Other plants belonging to this family are recorded as containing over 10 % tannins; among these are a number of *Acacia, Albizia, Cassia* and others (Rizk, 1986).

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تأثيرالمتغيرات الموسمية على نواتج الأيض الثانوية والقيمة الغذائية لنبات النتش (كروتولاريا إيجيبتياكا)

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تهدف هذه الدراسة للتعرف على تأثير المتغيرات الموسمية على الأيض الثانوى والقيمة الغذائية لنبات النتش من العائلة البقولية بمنطقة وادى حجول عند الكيلو ١٥ من بداية الوادى خلال موسمى الصيف والشتاء ، وقد أظهرت النتائج أنخفاض القيمة العصارية لنبات النتش فى فصل الشتاء عنه فى فصل الصيف بينما أرتفعت نسبة كلا من الرماد النباتى، الكربوهيدرات الكلية والالياف والدهون الكلية، وقد اعطى الرماد أعلى قيمة له فى الصيف عنه فى الشتاء·

أظهرت النتائج ان محتوى النبات من عنصرى الكالسيوم والماغنسيوم خلال فصل الصيف قد وصلا لأعلى قيمة لهما لمواجهة التأثير الضار لعنصرى الصوديوم والكلوريد·

واحتوى النبات خلال فصل الشتاء على ١٤ حامضا أمينيا يدخل فى تركيب البروتين بنسب مختلفة من التركيزات وكان حامض الجلوتاميك اكثرهم تركيزا ، بينما احتوت عينات الصيف على ١٧ حامضا أمينيا وكان حامض الأرجنين اكثرهم تركيزا·

وأتضح من تقدير نسب المواد الفعالة لكل من النبات والبذور زيادة نسبة كل من الفلافونيدات و القلويدات الكلية بالبذور عنها فى النبات الكلى وكذلك خلال فصل الشتاء عنه فى فصل الصيف بينما كانت نسبة الصابونينات الكلية أعلى فى عينة البذور عنها فى النبات وخلال فصل الصيف عنها فى فصل الشتاء· وبتقدير نسبة التانينات الكلية اتضح زيادة نسبتها فى عينة النبات خلال موسم الصيف·

وأوضحت الدراسة أن القيمة الغذائية لنبات النتش عاليه فى الحالة الخضرية للنبات، بينما أظهرت النتائج ان نسبة كل من القلويدات والفلافونيدات والصابونينات وصلت لأعلى قيمة لها عند تكوين النبات للبذور، ولذلك يوصى بعدم تغذية الحيوان على النباتات فى هذه المرحلة حتى لا يتسبب نفوق الحيوان·