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# COMPARATIVE AND TAXONOMIC STUDIES ON FOUR *Trifolium* SPECIES IN EGYPT

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## ABSTRACT

The study was conducted on three wild species found in the north coast (Porg El Arab) area in Egypt and belong to the genus ((*Trifolium*). The wild species are: *T.esupinatum, T. fragiferum* and *T. repens,*

A comparative study was performed on the three wild *Trifolium* species and the cultivated one; *Trifolium alexandrinum.*. The study focused on the micro and macro morphological characters of leaves and seeds, the quality components, in addition to micro-elements. The research aimed to discriminate the genus *Trifolium* using morphological characteristics and surface properties of the leaflet and the seed using scanning electron microscope and anatomical characteristics to be used as criteria and divisional signs to identify those species in the future.Morever, the aim of this study was to document this wild species in the Egyptian flora and to study the possibility to cultivate them as a fodder plants.

Assessment of the three wild species in terms of forage quality values was compared with Egyptian clover (*T. alexandrinum*). The study showed that the wild species have a high content of protein, carbohydrate and crude fiber and ash, as well as the content of elements (N.P.K).

The study recommended the possibilities of sowing the wild *Trifolium* species (alone or intercropping with other forage legumes and grasses) in new broad pasture areas to increase the animal resources in Egypt.

***Key words****: berseem , clover, crude protein, fiber , morphology, SEM ,Trifolium, Trifolium alexandrinum, T. fragiferum, T.repens, T. resupinatum.*

## 1. INTRODUCTION

Clovers are considered a very important part of grassland crops among the Legume family. There is some confusion concerning the number of species which the genus *Trifolium* has been included. Evans, (1976) mentioned that the genus includes more than 250 species of which twenty are of considerable agricultural importance. While, Zohary and Heller, (1984) reported 237 species within this genus. Boulus (1999) indicated that about 240 species of clover are found in Egypt.

Large areas of reclaimed soils are not available to growing with Berseem clover but able to be cultivated alone by some wild *Trifolium* species or intercropping with other crops such as *Trifolium repens, Trifolium fragiferum* and *Trifolium resupinatum*. Berseem Clover *(Trifolium alexandrinum* L.) is an important winter fodder crop of irrigated areas in Egypt. It is a multi-cut crop which produces nutritious and palatable fodder for the cattle. Fodder yield of berseem is

low in Egypt due to the low yield potential of cultivars being grown by the farmers and limited area in the Delta. Berseem is a high-quality forage characterized by high concentration of nutrients, primarily proteins (15-25% DM), minerals (11-19%) (Sharma and Murdia 1974). *Trifolium repens* is a nutritious forage, rich in protein, minerals and soluble carbohydrates. Compared to other temperate forages, it has relatively low levels of fiber and lignin (Thomson, 1984 and INRA, 2007).

Surface sculpturing (by using Scanning Electron Microscope (SEM) technique) may aid in solving problems of identity or relationship concerning taxa at various levels (Werker, 1997).

The seeds of Leguminosae in particular are highly varied in its shape (Kopooshian and Isley, 1966). The great variations in the morphology and the different ornamentations of the seed coat

support the study of taxa delimitation and may solve and facilitate many taxonomic problems. Vaughan (1968) suggested that the structure of the mature seeds, especially the coat is considered the more taxonomic useful information.

The SEM examinations of seed surface features could be applied in taxonomy and there are many characters (*e.g.* seed coat) could be used to characterize groups of related species, genera or taxonomic categories up to the sub-family levels. Some characters of the micro-morphology and orientation of epicuticular wax crystalloid are surprisingly of high systematic significance (Barthlott, 1981). Kadry (2002) suggested that the macro and micro morphological characters of seeds (shape, color, size, epidermal cell shape, anticline wall boundaries and the outer periclinal cell walls) were used for species identification.

The objectives of this study were to distinguish the taxonomic relationship between the studied *Trifolium* species and to compare the nutritive values of these species to increase the animal feeding resources.

## 2. MATERIALS AND METHODS

In this study, four species of the genus *Trifolium* were examined (Table 1). These species were planted in Medicinal and Aromatic Plants Research Department, Horticultural Research Institute, Agricultural Research Center, Dokki, Giza. The fresh leaves and seeds of each species were used in this study. The detailed surface scan features were examined by using Scanning Electron Microscope (SEM) with different magnifications. Scanning was carried out by JEOL- JSM T 100 Model Scanning Electron Microscope, Central Laboratory National Information and Documentation Center (NIDoC), Dokki, Giza, Egypt.

Micro and macro-morphological characters of leaf and seed surface sculptures were used to explore the relationship between the studied *Trifolium* species. In addition to, the data obtained from analyzing the chemical components of these species from protein, crude fiber, carbohydrate, and ash percentages, and N.P.K. percentages.

For chemical determinations, sample of 200 g bulk plants per species were fine powdered and wet digested according to Chapman and Pratt (1961). Nitrogen percentage was determined in plants by wing microkjelhal methods. Phosphorus and potassium percentages were determined by using the procedure described by A. O. A. C. (1990).

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## 3. RESULTS AND DISCUSSION

The species under consideration were studied and the results were gathered according to the following aspects:

Macro and micro-morphological descriptions of leaf (upper and lower surface) (Table 2 and Fig.1), and seed surface (Table 3 and Fig.2) for each species.

**1- Macro-morphological descriptions of leaves.**

The results revealed that there are three different shapes and sizes of leaflets as follows (Table 2):

i. Oblong to elliptic shape characterized the leaflets of *T.alexandrinum*. The leaflet length

**Table (1): The studied species, growth habit and the collection regions.**

| No. | Species | Habit | Region |
|---|---|---|---|
| 1. | *Trifolium alexandrinum* L. | Cultivated | Field in Giza |
| 2. | *Trifolium fragiferum* L. | Wild | North Coast (Porg El Arab) |
| 3. | *Trifolium repens* L. | Wild | North Coast (Porg El Arab) |
| 4. | *Trifolium resupinatum* L. (=*T. suaveolens* ) | Wild | North Coast (Porg El Arab) |

**Table (2): Macro and micro-morphological descriptions of the leaves of the studied species.**

| Species / Character | | *T.alexandrinum* | *T. fragiferum* | *T. repens* | *T. resupinatum* |
|---|---|---|---|---|---|
| **Leaf** | **Color** | Green | Green with prominent veins | Green with white ring | Green |
| | **Texture** | Appressed hairy or glabrescent | Glabrous or sparsely hairy | Glabrous | Glabrous |
| | **Petiole (cm)** | 3-7 | 8-10 | 16-18 | 8-10 |
| | **Arrangement** | The lower alternate& upper mostly opposite | Alternate | Alternate | Alternate |
| **Leaflets** | **Margin** | Serrate | Spinulose-serrate | Denticulate-serrulate | Serrate - |
| | **Shape** | Oblong to elliptic | Obovate To Elliptic | Broadly obovate to orbicular | Obovate to elliptic |
| | **Apex** | Mucronate | Obtuse or retuse | Obtuse or apiculate | Obtuse or emarginate |
| | **Base** | Obtuse | Cuneate | Obtuse | Cuneate |
| | **Length cm** | 1.5-4.6 | 0.6-1.9 | 1- 2.5 | 1- 2.1 |
| | **Width (cm)** | 0.5-1.9 | 0.4-1.2 | 0.8-1.7 | 0.5-1.5 |
| **Petiolate mm** | | Sessile | 2-2.3 | 1.4-17 | 3-3.2 |
| **Upper epidermis** | **Type of stomata** | Anomocytic | Anomocytic | Anisocytic &anomocytic | Anomocytic |
| | **Stomatal leveling** | Semi-depressed | Semi-depressed & depressed | Depressed & superficial | Semi-depressed |
| | **Type of trichomes** | Non-glandular (Hairs) | — | — | — |
| | **Trichome ornamentation** | Micropapillate (Verrucose) | — | — | — |
| | **Sculpture** | Weak reticulate | Colliculate | Ocellate | Areolate - reticulate |
| **Lower epidermis** | **Type of stomata** | Anomocytic | Anomocytic | Anomocytic | Anomocytic |
| | **Stomatal leveling** | Superficial | Depressed | Superficial | Superficial & raised |
| | **Type of trichome** | Non-glandular (Hairs), longer, Present on all Surface | Non-glandular (Hairs) Abundance, only Present on midrib | — | — |
| | **Trichome ornamentation** | Micropapillate (Verrucose) | Micropapillate (Verrucose) | — | — |
| | **Sculpture** | Weak reticulate | Reticulate | Rugose | Rugulose |

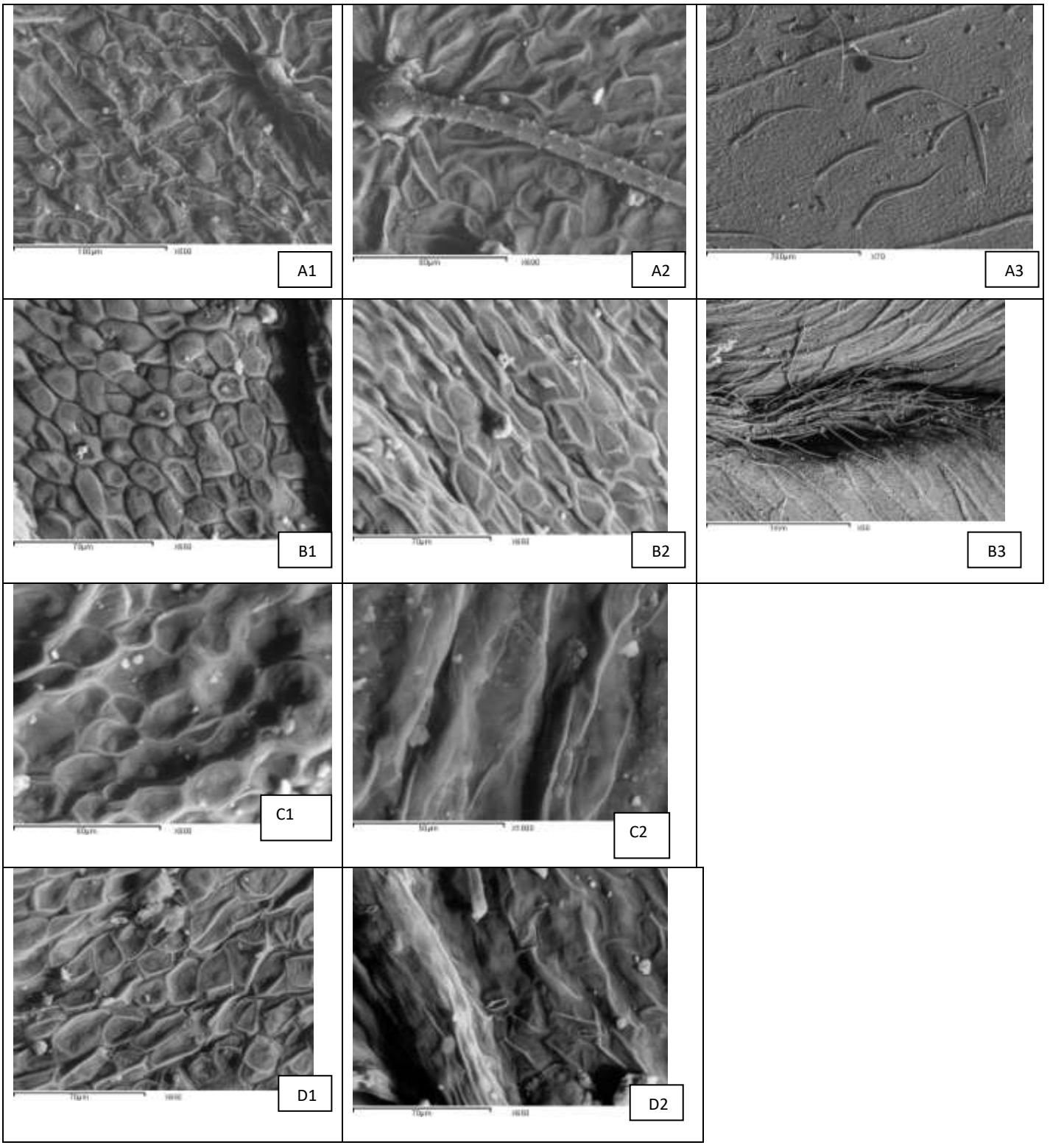


**Fig. (1): SEM on leaf epidermis; (1)upper surface,(2) lower surface and trichomes of surface (3)**
**A)** *Trifolium alexandrinum* **B)** *T. fragiferum* **C)** *T. repens* **D)** *T. resupinatu*

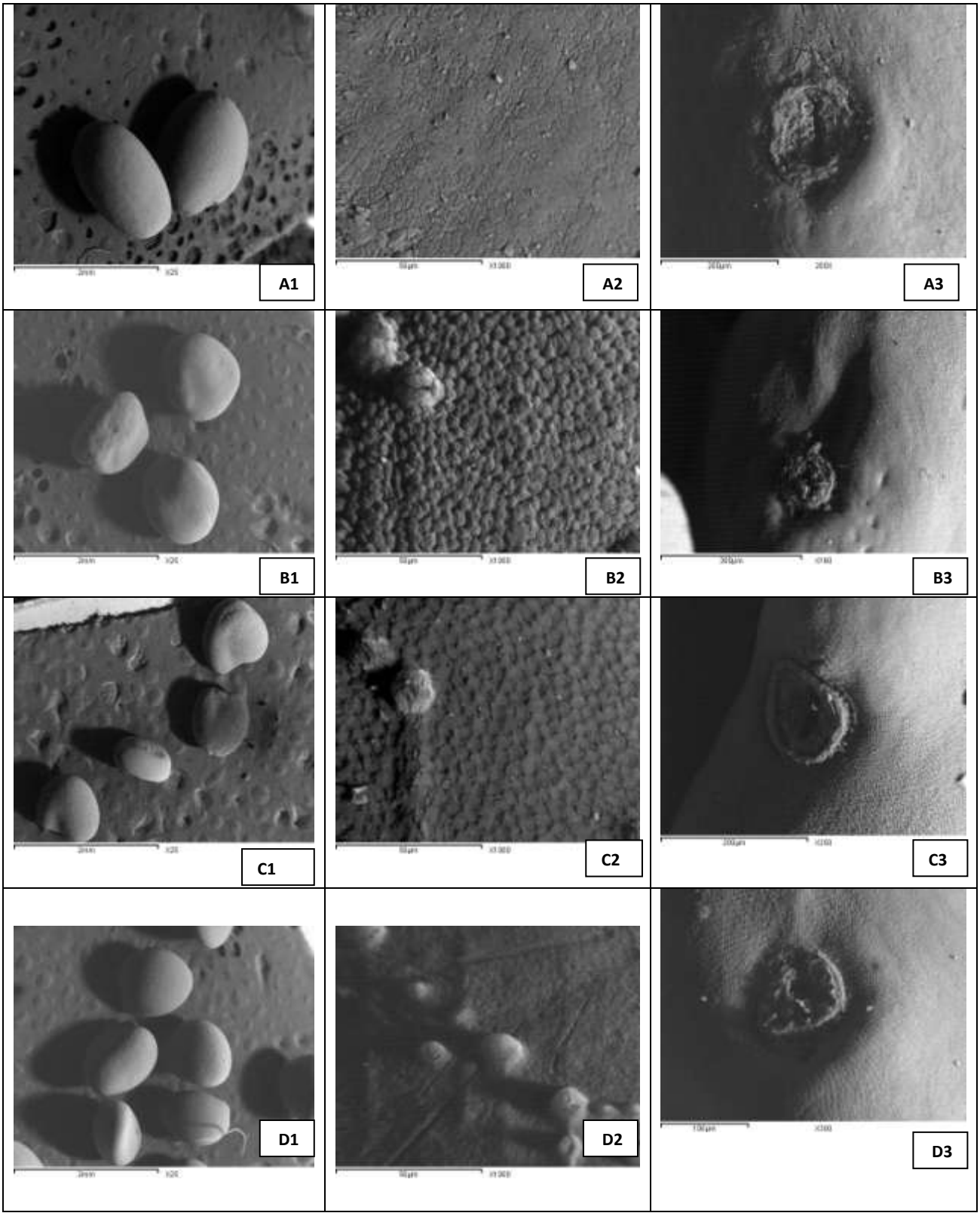


**Fig. (2): SEM of seed; shape (1), surface sculpture(2) and hilum (3)**
**A)** *Trifolium alexandrinum*, **B)** *T. repens*, **C)** *T. fragiferum* **and D)** *T. resupinatum*

**Table (3): Morphological descriptions of the seed of the studied species.**

| Species Character | | *T. alexandrinum* | *T. fragiferum* | *T. repens* | *T. resupinatum* |
|---|---|---|---|---|---|
| **Shape** | | Ovate or elliptic | Reniform | Ovoid | Ovoid |
| **Seed No./ pod** | | 1 | 2 | 3-4 | 1 |
| **Color** | | Yellow to brown | Light brown with dark brown flecks | Brown | Brown |
| **Length (mm)** | | 2-2.5 | 1-1.5 | 1-1.3 | 1.2-1.5 |
| **Width (mm)** | | 1-1.5 | 1-1.2 | 1-1.1 | 1-1.2 |
| **L X Wmm$^2$** | | 2-3.75 | 1-1.8 | 1-1.43 | 1.2-1.8 |
| **Grade** | | Large | Medium | Small | Medium |
| **Hilum** | | Circular with slit – like opening basal, | subcircular with narrowly oblong opening**.** lateal | Circular with ovate opening, basal, | Ovate with circular opening, basal, |
| **Surface pattern** | | Ruminate | Verrucate | Verruculate | Globulate – Tubercate |
| **Anticlinal walls** | **Gonal No.** | 4-6 | 4-5 | 4-6 | 5 many |
| | **Epidermal cell** | Elongate in one direction | Elongate in one direction | Irregular | Elongate in one direction |
| | **Relief of cells boundaries** | Flat, straight | raised, straight or sinuous | Channeled straight or sinuous | Flat,or slightly raised, straight |
| **Outer periclinal cell wall surface** | | Flat | Flat or concave with some rounded projections | Domate with few irregular projections irregular striation | Flat with many aggregate bodies |

**Grade:- Large: equal or more than 2 mm$^2$, Medium:2 > M > 1.6 mm$^2$, Small = less than 1.6 mm$^2$**

averaged 1.5-4.6 cm, with mucronate apex and obtuse base.

ii.Obovate to elliptic shape characterized the leaflets of *T. fragiferum* and *T. resupinatum* with leaf length ranged between 0.6-1.9 cm, with cuneate base. Obtuse or retuse apex in *T. fragiferum* and obtuse or emarginate apex in *T. resupinatum*.

iii.Broadly obovate to orbicular shape in *T. repens*, the leaflets length 1- 2.5 cm, with obtuse or apiculate apex and cuneate base.

Moreover, all these leaves of studied species were alternate and petiol present. Based on the leaf texture, the studied species split into two categories; glabrous leaves in two species; *T. repens* and *T. resupinatum* and glabrescent or sparsely hairy leaves in the other two species.

**2- Micro-morphological descriptions of leaf**

A- Upper epidermis:

There are four sculpture patterns of the upper epidermal surface of leaf of the studied species (Table 2 & Fig. 1):

- Weak reticulate as in *T.alexandrinum*. Colliculate as in *T. fragiferum*.
- Ocellate as in *T. repens*
- Areolate - reticulate as in *T. resupinatum*.

B- Lower epidermis:

There are three sculpture patterns of the lower epidermal surface of leaf of the studied species (Table 3 & Fig. 1):

- Reticulate as in *T. fragiferum* and appearance weak reticulate as in *T.alexandrinum*.
- Rugose as in *T. repens.*
- Rugulose as in *T. resupinatum*.

**3-Macro-morphological descriptions of seed** (**Table 3 & Fig. 2**)

**i) Shape and grade**

The seeds of the studied species have three varied shape; ovoid in *T. repens* and *T. resupinatum*, ovate or elliptic in *T. alexandrinum* and reniform in *T. fragiferum.*

The grade exhibits more variability. Mature seeds of all examined species are ranged in size from 1-2.5 x 1-1.5 mm (Length x Width). The smallest seeds were those of *T. repens*, while large seeds as in *T. alexandrinum* and remaining two species

have medium sized seeds.

**ii) Color**

In all the studied species, the seed color varies from brown (in *T. repens* and *T. resupinatum*), yellow to brown (in *T. alexandrinum*) to light brown with dark brown flecks (in *T. fragiferum*).

**4- Microrphological descriptions of seed**

**i) Sculpture pattern of seed surface**

1. Ruminate: as in *T. alexandrinum*
2. Globulate – Tubercate as in *T. resupinatum*.
3. Verrucate: as in *T. fragiferum.*
4. Verruculate: as in *T. repens*.

**ii) The anticlinal cell walls**

**1. Number of gonals**

a) Five to Polygonal: as in *T. resupinatum.*

b) From 4-6: as in *T. alexandrinum* and *T. repens*.

c) From 4-5: as in *T. fragiferum*.

**2. Relief of cell boundaries**

a. Flat: as in *T. alexandrinum.*
b. Channelled: as in *T. repens*.
c. Raised: as in *T. fragiferum*
d. Slightly raised:or flat as in, *T. resupinatum*
e. Straight or sinuous: as in *T. repens*.and *T. fragiferum*
f. Straight: as in *T.resupinatum* and *T. alexandrinum*.

**iii) The outer periclinal cell wall surface**

a. Flat: as in *T. alexandrinum.*
b. Flat with many aggregate bodies: as in *T. resupinatum.*
c. Flat or concave with some rounded projections: as in *T. fragiferum.*
d. Domate with few irregular projections and irregular striation: as in *T. repens*.

**iv) The hilum**

a. Circular with slit – like opening, basal: as in *T. alexandrinum* and with ovate opening, basal: as in *T. repens*.
b. Subcircular with narrowly oblong opening**.** Lateral: as in *T. fragiferum.*
c. Ovate with circular opening, basal:as in *T. resupinatum*.

**Forage quality**

Forage has an important role in ruminant nutrition in terms of providing energy, protein and minerals, as well as fiber for chewing and rumination. In the Mediterranean areas pastures represent the most important forage resource. Clovers are primarily used for pasture, but also for stored feed. Grazing is more convenient and costs less than half the amount of stored feed, which has additional costs associated with hay and silage harvesting and feeding. (Ball *et al.,* 1996).

The high nutritive value of clover allied to its high intake characteristics. Forage crops production is very important for successful animal production (Dabkeviciene *et al.,* 2008). Species had a highly significant effect of quality components, crude protein, carbohydrate, crude fiber and ash percentages (Table 4). In addition, the percentages of macro-elements (N, P and K%) in plant tissues were significantly varied according to the species of *Trifolium* used. The maximum protein and carbohydrate percentages were obtained by *T. alexandrinum*; 16.56 and 66.82%, respectively. The highest protein percentage was related to high content of N (2.64 %) in berseem plant tissues. Santis *et al.* (2004) suggested the best stage of harvest management per cut, six$^{th}$ internode elongation, for obtaining relatively high yields of forage with high nutritive value in berseem clover.

*T. repens* was rich in protein content (13.9%) and N (2.22%), these results agreed with McGraw

**Table (4): Quality of components and NPK elements as a percentage of dry matter of four *Trifolium* species.**

| Species | As a percentage of dry matter | | | | | | |
|---|---|---|---|---|---|---|---|
| | **CP** | **Carbohydrate** | **CF** | **Ash** | **N** | **P** | **K** |
| *Trifolium alexandrinum* | 16.56$^{a}$ | 66.82$^{a}$ | 29.58$^{b}$ | 11.74$^{b}$ | 2.64$^{a}$ | 0.27$^{c}$ | 3.37$^{a}$ |
| *T. repens* | 13.93$^{b}$ | 47.89$^{c}$ | 33.74$^{a}$ | 13.78$^{a}$ | 2.22$^{b}$ | 0.27$^{c}$ | 2.29$^{c}$ |
| *T. fragiferum* | 12.31$^{c}$ | 59.48$^{ab}$ | 28.97$^{bc}$ | 11.26$^{b}$ | 1.96$^{c}$ | 0.32$^{b}$ | 2.97$^{b}$ |
| *T. resupinatum* | 13.55b$^{c}$ | 62.78$^{a}$ | 27.97$^{c}$ | 13.18$^{a}$ | 2.16$^{bc}$ | 0.45$^{a}$ | 2.94$^{b}$ |
| Mean | 14.09 | 59.24 | 30.06 | 12.50 | 2.45 | 0.33 | 2.89 |

**In each column means followed by similar letters are not significantly different at 5% level.**
**CP=Crude protein, CF= Crude fiber, N= Nitrogen, P=Phosphors and K= Potassium contents in plant tissues.**

*et al*. (2004). *T. repens* recorded minimum content of carbohydrates (47.89%) with maximum content of crude fiber and ash (33.74 and 13.78%, respectively) (Table 4). White clover recorded high levels of crude protein, soluble carbohydrates, minerals (especially calcium, phosphorus, magnesium) and low levels of structural carbohydrates and lignin (Ulyatt *et al*., 1977 and Thomson, 1984). *Trifolium repens* L. is considered an effective way of improving pasture production and soil N status in many parts of the world (Gibson and Cope, 1985 and Ledgard and Steele, 1992).

Persian clover *(T. resupinatum*) recorded high percentage of crude protein, carbohydrate and P content; 13.5, 62.78 and 0.45 %, respectively with the lowest percentage of crude fiber (27.97%). *T. resupinatum* provides a high-quality forage (dry matter, crude protein, crude fiber, P, K, Ca and Mg) for animals throughout the growing season (Tekeli *et al*., 2003).

*T. fragiferum* had the lowest N content (1.96%) among all the species under study. Moore *et al*. (2006) recorded that the percentage of crude protein of *T. fragiferum* ranged from 18 to 23% but McGraw *et al*. (2004) concluded that selection for forage quality only; the native legumes did not fare as well as introduced species, having less crude protein and more cell wall fiber. The high percentage of (K) may be pointed to adaptability and tolerant to some ecological stresses. Xingyu Jiang *et al*. (2010), reported that potassium is a crucial feature for plants under high-salt conditions. Proper harvest timing is important in determining forage quality. The crop should be harvested before blooming period to maintain high forage quality. For *T. repens, T. fragiferum and T. resupinatum,* harvests in the late vegetative or early bud stage get higher protein content compared with the other harvest periods. The nutritive value of clovers may be influenced by changes in the nutrient concentrations of morphological fractions as a consequence of cutting treatment.

In conclusion, the severe shortage of fodder and the needs to increase the livestock resources especially in the presence of some cultivated species of the genus *Trifolium* in Egyptian flora encourage plant breeders to study these species. The concentrations of the mineral contents also reflect the mineral status of the soil and the supply of the fertilizer nutrients, and are influenced by the species of the forage crops. The high-quality forage may be obtained from these clovers cut at all the growing stages. According to forage quality components, these clovers can be sown in Egypt as well as berseem and alfalfa climate conditions. There is a possibility to expand growing of the three wide *Trifolium* species, in extended pastures of the newly reclaimed soils either individually or intercropping with some grasses to improving the forage yield and quality.

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## دراسات تصنيفية مقارنة بين أربعة أنواع من البرسيم بمصر

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مركز البحوث الزراعية – الجيزة - مصر

**ملخص**

أجريت هذه الدراسة على ثلاثة أنواع وجدت بصورة برية في الساحل الشمالي (منطقة برج العرب) و تنتمي لجنس البرسيم (*Trifolium*) و مقارنتها مع النوع المنزرع *Trifolium alexandrinum*.

وكانت الانواع البرية هى: *T. fragiferum , T.resupinatum , T. repens*

وتركزت الدراسة على الصفات المورفولوجية وخصائص سطحى الورقة والبذرة باستخدام الميكروسكوب الإلكتروني الماسح والمحتوى البروتينى والكربوهيدراتى و الألياف الخام والرماد بالاضافة الى المحتوى من العناصر N.P.K.

*ويهدف البحث الى:*

تمييز انواع جنس *Trifolium* التي تم دراستها باستخدام الصفات المورفولوجية وخصائص المسح السطحى للورقة والبذرة باستخدام الميكروسكوب الماسح الألكترونى والصفات التشريحية بهدف استخدامها كمعايير ودلائل تقسيمية للتعرف على تلك الانواع مستقبلا

توثيق انواع جنس*Trifolium* التي درست والنامية فى مصر ودراسة امكانية استخدامها كنباتات علف

تقييم الانواع البرية من حيث قيمتها العلفية مقارنة مع البرسيم المصرى حيث أظهرت الدراسة أن الانواع البرية ذات محتوى عالى من البروتين والكربوهيدرات و الألياف الخام والرماد بالاضافة الى المحتوى من عناصرN.P.K .

ولذا توصي هذه الدراسة بزراعة (*T. fragiferum, T. resupinatum, T. repens*) في مناطق رعوية واسعة في الأراضي الجديدة حديثة الإستصلاح إما بصورة منفردة أو في زراعات التركيب المحصولي مع محاصيل نجيلية أخري من أجل زيادة الإنتاجية و القيمة الغذائية لمحصول العلف الناتج بما ينعكس علي زيادة الثروة الحيوانية في مصر.

**المجلة العلمية لكلية الزراعة – جامعة القاهرة – المجلد (64) العدد الثانى (أبريل2013):152-160.**