The Response of *Galega officinalis* Plant to Different Nitrogen Sources and their Effect on Active Ingredients and Biological Activity

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Abstract: *Galega officinalis*, goat′s rue, a plant native to Euro-Asia and North Africa was introducing to Egypt as an objective for introducing important medicinal plants. The plant claimed to be useful in diabetic treatment, reduce weight and as anti platelet aggregation. Plant density and different nitrogen sources (organic, bio and chemical fertilizers) were tried to evaluate their effect on growth, yield, active ingredient, and its biological activity as anti diabetic. The results revealed that wide propagation distance produced higher growth and yield than the narrower one. The mineral nitrogen proved to be the effective source followed by compost then cattle manure on growth and yield of leaves, whole plant, flower, and sucker number. Total alkaloid was determined in leaves and it fluctuated with no clear trend, however was higher in sample taken at June. The fertilization had no effect on alkaloid accumulation.
Goat′s rue total alkaloid showed the highest reduction percentage in blood glucose level after 2 hours in diabetic rats.
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Key words: Galega, Nitrogen sources, rates, planting distance, alkaloid, anti-diabetic

1. Introduction:

Galega officinalis, commonly known as goat`s rue, French lilac, Italian flitch or professor weed, is an herbaceous plant in the Faboideae subfamily. It is native to the Middle East, but it has been neutralized in Europe, western Asia and western Pakistan. The plant has been extensively cultivated as a forage crops, an ornamental, a bee plant and as green manure (lasseigne, 2003). Its name drives from gale "milk" and ega "to bring on" as Galega used as a galactogogue in small domestic animals.

Galega officinalis has been known since the middle ages for relieving the symptoms of diabetes mellitus, upon analysis it turned out to contain guanidine a substance, that decrease blood sugar by decreasing insulin resistance, Pundarikakshudu *et al.* (2001) reported the anti- bacterial activity of *Galega*. ethanolic extract both gram negative and positive bacteria. Peiretti and Gai (2006) reported the nutritive value, fatty and amino acids content of Galega herbage during growth cycle and in regrowth in samples collected at progressive morphological stages and during regrowth.

Other uses of Galega were indicated as its usefulness in weight reduction (Palit *et al.*, 1999), inhibition of platelet aggregation (Atanasov and Spasov, 2000), against plaque (Bunney, 1986) in foot bath and ointment to hasten skin healing after surgery (Pandarikakshudu *et al.*, 2001). It increases and stimulates milk lactic secretion and milk production in animals (Gonzalez-Andres *et al.*, 2004)

In a project deals with plants have antidiabetic effects, this plant was introduced to Egypt, where different agricultural treatments will be tried to adapt this plant to Egyptian conditions.

This investigation deals with using different fertilization rates and kinds and their effect on bio-mass production, alkaloid content and its biological activity as anti– diabetic.

2. Material and Methods

Seeds of *Galega officinalis* were directly sown in the field on 20 March 2008 and germinated on 28 March. Seven treatments beside control were performed as follows;

1. control,
2. cattle manure 20 m^3/ acre,
3. Rhyzobacterin + 15 m^3 compost/ acre
4. Phosphorin + 15 m^3 compost / acre
5. Compost 20 m^3/ acre
6. Ammonium nitrate 100kg/acre(33.5%N)
7. Ammonium nitrate 200kg/acre (67%N).

The propagation was done on rows 60cm apart at a distance of 20 and 40cm between plants. The treatments were arranged in complete randomized blocks, each one replicated 3 times in an area of $3m^2$ (3 x1 m).

Different fertilizers of varied sources were added four times on May 16, June 4, October 17 and November 27. the compost and cattle manure added before cultivation through land preparation.The bacterial extract was added at a rate of 10 ml diluted to 1 liter water where each ml of the bacterial extract contains 10^{-6} bacterial cells.

The compost obtained from sekem company (Cairo – Belbeis Desert Road, El-Horreya, Cairo) was added and analyzed; its values were presented in Table (1)

Table (1) chemical Analyses of Compost.

Constituent	values
Bulk Density kg/m^3	510
Moisture Content %	18.2
Electrical conductivity dS/m	9.65
pH	7.6
Total Organic Carbon %	24.6
Total Organic Matter %	42.41
C/N Ratio	18.22
NH_4-N, mg/kg	880
$N0_3$-N, mg/kg	450
Total Phosphorus %	1.6
Av. Phosphorus mg/kg	410
Total Potassium %	2.3
Av. Potassium mg/kg	620
Trace Element (ppm)	
Fe	960
Zn	280
Mn	210
Cu	140
Nematodes	nil
Weeds Germination	nil
Parasites	nil
Pathogenic	nil

Cattle manure was also analyzed and the data was compiled in Table (2)

Table (2): Analyses of cattle manure.

Weight of m^3 /kg	685
Humidity	6.19%
Total nitrogen	3.45%
Ammonia mg/kg	922.1
Nitrate mg/kg	74.3
Total phosphorus %	0.53
Total potassium %	2.06
Organic matter	63.14
Organic carbon %	61.11
Iron mg/kg	-
Mn mg/kg	188.6
Cu mg/kg	55.3
Zn mg/kg	875.6

The first record for vegetative growth was obtained on 15 th June 2008 and the same growth

parameters were recorded in different dates; 24th July, 6 th September, 27 th November, 31 th December 2008 and 12 th March 2009.

The following measurements were recorded: plant height over ground in cm, sucker's number, whole plant weight fresh and dried, and leaves weight fresh and dried in grams per plant, and flowers fresh and dry weight.

The plants were cut off 10cm over ground at the end of September, growth was continued, where 3 samples were taken on; November, December 2008 and on March 2009.

Growth parameters

The growth aspects measured were compiled in Tables 3, 4, 5, 6 and Fig.1 for the samples taken through the growth season and regrowth.

Extraction of crude alkaloid

About 200g dried powdered leaves of *Galega officinalis* was extracted with 5% Hcl in ethanol different times till exhaustion. The combined extract was evaporated till 10 ml and then pH was adjusted to 11 by adding NH_4OH.

The total extract is partitioned between chloroform and water. The chloroform layer was concentrated till dryness (total alkaloids), and then weighed and total alkaloid as percentage was calculated according to the method of Edeoga *et al.* (2005).

Ethanol extract

About 500g dried powdered leaves of *Galega* was percolated with ethanol 70% (3L, five times). The combined ethanol extract was evaporated till dryness.

The crude alkaloid, standard galegine (metformin) as wells as ethanol extract were applied on TLC aluminium sheet with the following solvent systems:

1- Chloroform- MeOH (9:1). 2- Benzene: EtOAc (8:2). 3- Benzene: EtOAc (9:1).

The plates were sprayed with Dragendorff reagent to visualize the color of alkaloid; the different R_f values were presented in Table (7).

Acute toxicity of crude alkaloid

Animals and diet:

Male Albino mice weighing from 20-25g were obtained from Animal House of National Research Centre, Egypt. The mices were fed on a basal pellet diet. All animals were maintained in plastic cages with free access to tap water.

Experimental design

Acute toxicity assay of total alkaloid studied on eight groups of mices, each group contained 6 mices. The test was carried out among equal sized groups of lethality in mice receiving progressively increasing oral dose levels of the total alkaloid (250, 500, 750, 1000, 1250, 1500, 1750, 2000 mg/kg b.wt).

After administration of alkaloid, animals were observed closely during first 48h and then every 12h for one week.

The experimental work on mices was performed with approval of the animal care experimental committee, National Research Centre, Egypt, according to the guidance for care and use of laboratory animals.

Oral glucose tolerance test (OGTT)

Male Albino rats were fasted overnight (at least 12h). They were divided into three groups containing six animals each. Control rats (group 1) were given 1 ml distilled water. Metformin (as a reference drug) and galega alkaloids at concentration of 200 mg/kg (b.wt.) were administered orally using a syringe to the second and third groups. After half an hour of extract administration, the rats of all groups were orally administrated with 2g/kg b.wt of glucose. Blood samples were collected from the retro-orbital plexus just prior to glucose administration and 30, 60 and 120 min, after glucose administration.

Loading-Plasma was separated and blood glucose levels were measured immediately by the glucose oxidase method (Trinder, 1969).

Chromatographic investigation:

Chromatographic investigation was performed to check the occurrence of alkaloid either in ethanol extract or precipitated as crude alkaloid in comparison with metformin which is considered as reference. Different solvent systems were tried, where Rf values on TLC were present in Table (7).

3. Results

Six samples were taken from the experiment including the different growth parameters beginning 15th June and then with intervals of 45 days.

The first sample taken on 15th June 2008 illustrated in Fig. (1), the data obtained indicate that all treatments increased growth parameters studied. The most potent treatment was ammonium nitrate in its high rate. Whole plant weight was 100.68 against 23.96 gm in control. These finding was true at the two spacing distance 20 and 40cm. As to compare between the two spacing distance, 40cm gave more biomass specially with treatments of compost (98.95) and the two rates of ammonium nitrate (67.91 and 119.18 gm) which produce more vegetative growth than plants propagated at 20cm space.

The data of the second sample (Table, 3) reveals that all the parameters increased either in

plant height or weight as the plant progressed in age. When the different treatments were considered, it was found that cattle manure (105cm) and the high rate of ammonium nitrate (108.76cm) produced taller plants. On the other hand the vegetative growth was vigorated by cattle manure (379.40g) and compost (320g) treatments, with plants cultivated at 20cm space distant.

With regard to plants cultivated at 40cm, cattle manure produced the tallest plants (106.77), overall the treatments, but the vegetative growth was stimulated by compost treatments and ammonium nitrate only.470.0 and 405.67g/plant respectively.

The third sample taken represent the progress occurred through growth season Table (4), meaning, increase in all parameters studied over two previous samples. Maximum length of plants was produced from plants fertilized with compost and ammonium nitrate 200 kg/acre, (100.43 and 100cm) respectively.

Suckers number was the highest in treatment of cattle manure followed by ammonium nitrate 100 kg/acra., 54 and 39 suckers / plant respectively.

Cattle manure and ammonium nitrate in its high rates produced the highest fresh weight or biomass (whole plant). The same finding was reported with leaves fresh and dry weights. As to consider the wider distance of cultivation all the fertilization treatments vigorates the whole plant and leaves fresh weight over the control, due to treatments of ammonium nitrate, compost and rhizobacterin which produced the highest yield.

The fourth sample represents the regrowth obtained after cutting 10cm over ground and fertilized as previously performed then left to grow. With this condition which can be described as the re-growth of another cut, treatments of compost, and cattle manure increased plant length to nearly the same length (56cm) but lower than the previous two samples taken on July and Septemper. Compost highly increased whole plant fresh weight to reach 1011.20 gram/plant followed by the two rates of mineral nitrogen, 700.67 and 885.10g/plant, respectively.

Wider distance and ammonium nitrate (the highest rate) synergize the production of whole plant fresh weight to double fold the plants propagated at the narrower distance 2252.0gm/plant and consequently the leaves fresh weight 1716.87 gm and dry weight437.8 gm., Table (5)

The fifth sample was taken on 31-12-2008 75 days old from cutting .Fig. (2) clearly represents the variation in vegetative parameter due to fertilization treatments and propagation distances. All the growth parameters increased than the sample taken at the end of November regardless the low temperature prevail at this time. Treatments of compost and mineral nitrogen in the lower dose greatly increased plant length, whole plant fresh weight, leaves fresh and dry weights with plants cultivated at the narrower distances however wider distance produced lighter yield, and shorter plants.

The last sample taken from plants (75days old, Table (6) showed taller plants than that of the previous samples. No effect of the different treatments was revealed on plant height as control plant produced the tallest ones except with ammonium nitrate in its lower dose treatments. Cattle manure affects sucker no. greatly in wider distance of cultivation while compost treatment increases the no. of suckers in the narrower cultivation distance.

With respect to the effect of fertilization treatments on whole plant fresh weight, only compost stimulates this aspect on plants propagated at 20 cm. In plants propagated at 40 cm distance, all fertilization treatments increase whole plant fresh weight and leaves fresh and dry weights, the maximum growth yield was produced as an effect of ammonium nitrate in its lower rate.

A glance on all the samples taken, it was found that ammonium nitrate in its two rates added was the treatment of choice to increase the vegetative parameters studied on goats rue plants. Compost and or cattle manure came in the next order. In this respect compost produces higher yield or biomass especially in sample taken at low temperature (on December) as it is hydrolysable during the hottest dates and then releases its content in winter. The wider distance is the most suitable for propagation than that of 20cm irrespective of the high yield obtained with narrower one due to the high density of plants per acre.

Chromatographic analysis:

The chloroform and alcohol extract beside the standard metformin were applied on TLC plates in a trial to separate the alkaloids. In all the solvents applied one red spot representing the galegine or metformin was detected and has the Rf values represented in the following Table (7).

It was found that solvent I was the best to separate and detect the galegine alkaloid from all other plant components.

Determination of alkaloids:

Alkaloids were determined as total in the leaves of the different treatments according to the method of Edeoga *et al.* (2005), where it was determined gravimetrically.

The total alkaloids as galegine were estimated as percentage in leaves and were compiled in Fig (3) where it could be concluded that alkaloids are higher in June, in younger organs (the plant is about 3 months old) then decreased towards maturation till November where the plant was cut

over the ground. Fertilization was added at the end of November and new growth was progressed. The alkaloid percentage again increased gradually but not reached the higher percentage observed in June.

From Table (8a) and Fig.(3) it was reveled that the maximum alkaloid content was accumulated through November, at sample taken after cutting and contain higher biomass and higher alkaloid content.

Biological studies: Acute toxicity

To use this plant as hypoglycemic remedy, toxicity must be estimated. In the present investigation, no death in animals in any groups administered crude alkaloid except the group administered 2000mg/kg body weight, in which one rat died. So this plant is safe till 2 gram/kg b.wt.

Glucose tolerance in rats

Administration of 2g/kg b.wt. glucose to normal rats increased serum glucose to 106.6 mg/dl after 60 min. in group I. The administration of metformin as a reference and extract of Goat's rue suppressed the elevation of serum glucose at 60 and 120 min.

Goats rue extract induced the highest reduction in blood glucose after 2 hours as illustrated in Fig. (4)

4. Discussion:

The demand for medicinal plants is related to the great culture significance attached to medicinal plants. The growing demand has not only resulted in increased hazard for over exploitation of wild population but also on increased interest of cultivation. Major interest of different factors affecting yield components and active ingredients is fertilization. Interest which is concentrated on nitrogen fertilization and the usage of different sources especially organic one to minimize using mineral sources, enormous researches are dealing with this factor on different medicinal plants. In August 2010 through the ICHS at Lisbon, (the International Conference of Horticultural Science). different sources of nitrogen and different levels (urea, ammonium nitrate ammonium sulphate), at 0, 180, 240, 300, 360 kg N/Ha were applied as treatments for two medicinal plants *leonotis* and *Artemisia*. The results proved that the plants treated reacted positively with addition of the three nitrogen sources with most of the treatment showing a significant increase in the fresh mass yield specially with the lowest level of fertilizer applied, in South Africa presented by Prinsloo *et al.*(2010). With an Iranian experiment dealing with chamomile using organic cultivation and bio fertilizer and their effects on growth, yield and essential oil content. Salehi *et al*. (2010) concluded that it seems that organic cultivation of German chamomile can consider as an alternative system for conventionally system in production of yield and essential oils. Ferreria *et al.* (2010) in Brazil observed a linear increase in biomass production according days after transplantation and the highest production occurred at 180 kg/ ha nitrogen in an experiment using different nitrogen rates of 0, 45, 90, 135, and 180 kg N / ha and different harvest ages of 152, 242, 332 and 428 days after transplanting of *piper aduncum* plants. Biesiada *et al.* (2007) on their work on *Calendula* proved that form and rate of nitrogen had a significant effect on chemical composition. Plants fertilized with urea had high level of phenolic compound than other mineral nitrogen sources. Rosemary plants treated with bio-fertilizer (*Azotobacter vinelandii*) through its different cuts, cause slight improvement in growth characters and its content of essential oil amounted to 0.75 % as revealed by Leithy *et al.* (2006). Combination of mineral and chemical fertilizers with Barvar phosphate biofertilizer caused the highest seed yield and mucilage content of *Plantago ovata* as described by Majid *et al.* (2007)

All the results reviewed revealed the beneficial effect of either mineral nitrogen or organic or biofertilization which coincided with the result obtained with this investigation. Organic fertilization provides nutrients to plants, improve the soil physical structure, increase water retention, reduces the erosion losses and favors the biological control and even are beneficial than mineral ones in reducing the residual effect of heavy metals and pollution

Hendawy and El- Gengaihi (2010) in comparative studies on the role of organic, bio and mineral fertilization of nitrogen and phosphorus found that mineral nitrogen gave quick and significant effect on Borage and Echium plants. Organic and bio fertilization however increase both growth characters and fixed oil but were in second rank after mineral ones which release quickly than organic ones like compost.

Dealing with distance of cultivation, Chaves *et al.* (2010) from Brazil concluded that biomass production was inversely proportional to the special arrangement, with the greater biomass production (1034.93 kg / ha) in narrower spacing, although no statistical difference was verified between distance (1m×1m) and (1.0 m ×1.5 m). The same response was observed for the production of essential oil of *Piper callosum.*

Table (3) **Mean values of some growth parameters of *Galega officinalis* under two different spacing as influenced by different fertilizer treatments (24/07/2008)**

	20 cm						40 cm					
Treatment	**Plant height (cm)**	**Sucker No.**	**Fresh weight whole plant (g)**	**Leaves fresh weight (g)**	**Leaves dry weight (g)**	**Flower fresh weight (g)**	**Plant height (cm)**	**Sucker No.**	**Fresh weight whole plant (g)**	**Leaves fresh weight (g)**	**Leaves dry weight (g)**	**Flower fresh weight (g)**
Control	***74.00***	***11.00***	***233.52***	***156.73***	***33.38***	***2.58***	***85.33***	***10.67***	***209.98***	***127.86***	***27.23***	***2.45***
Cattle manure	***105.00***	***14.10***	***379.40***	***236.33***	***50.34***	***3.31***	***106.77***	***11.00***	***209.79***	***114.58***	***24.41***	***9.23***
Rhizobacterin	***98.00***	***12.33***	***290.67***	***240.00***	***51.12***	***19.30***	***104.33***	***15.00***	***181.03***	***143.74***	***30.62***	***4.35***
Phosphorine	***78.67***	***13.33***	***262.17***	***181.87***	***38.74***	***2.20***	***85.00***	***11.33***	***198.13***	***102.19***	***21.77***	***5.40***
Compost	***93.33***	***12.00***	***320.00***	***219.67***	***46.79***	***5.47***	***95.33***	***17.00***	***470.00***	***332.21***	***70.76***	***8.82***
Ammonium nitrate 100kg/fed	***86.33***	***21.33***	***264.67***	***257.33***	***54.81***	***11.00***	***92.17***	***17.33***	***405.67***	***165.80***	***35.32***	***8.98***
Ammonium nitrate 200 kg/fed	***108.67***	***11.00***	***265.00***	***164.07***	***34.95***	***12.37***	***96.00***	***23.00***	***433.00***	***187.00***	***39.83***	***4.30***
L.S.D. (5%)	4.41	2.35	10.42	12.22	3.85	1.92	5.36	2.01	10.74	10.61	3.52	1.55
L.S.D. (5%) (20*40 cm)	4.11	2.15	9.6	11.03	2.93	1.41						

Table (4) **Mean values of some growth parameters of *Galega officinalis* under two different spacing as influenced by different fertilizer treatments (06/09/2008)**

	20 cm						40 cm					
Treatment	**Plant height (cm)**	**Sucker No.**	**Fresh weight whole plant (g)**	**Leaves fresh weight (g)**	**Leaves dry weight (g)**	**Pods weight (g)**	**Plant height (cm)**	**Sucker No.**	**Fresh weight whole plant (g)**	**Leaves fresh weight (g)**	**Leaves dry weight (g)**	**Pods weight (g)**
Control	***77.67***	***23.00***	***195.00***	***195.00***	***44.46***	***31.51***	***84.67***	***18.00***	***252.67***	***126.70***	***28.89***	***10.91***
Cattle manure	***91.67***	***54.43***	***234.77***	***234.77***	***53.53***	***18.07***	***77.33***	***27.10***	***278.87***	***186.80***	***42.59***	***16.57***
Rhizobacterin	***84.00***	***30.67***	***204.77***	***204.77***	***46.69***	***35.24***	***73.00***	***26.20***	***384.77***	***193.87***	***44.20***	***16.25***
Phosphorine	***90.20***	***15.87***	***148.27***	***148.27***	***33.80***	***13.33***	***72.87***	***27.33***	***321.67***	***244.00***	***55.63***	***20.45***
Compost	***100.43***	***27.67***	***185.43***	***185.43***	***42.28***	***17.53***	***80.67***	***42.67***	***369.33***	***207.87***	***47.39***	***27.53***
Ammonium nitrate 100kg/fed	***99.67***	***39.53***	***224.93***	***224.93***	***51.28***	***27.17***	***69.53***	***19.00***	***292.53***	***165.27***	***37.68***	***10.81***
Ammonium nitrate 200 kg/fed	***100.00***	***22.67***	***259.67***	***259.67***	***59.20***	***19.99***	***115.67***	***35.87***	***626.43***	***321.67***	***73.34***	***37.93***
L.S.D. (5%)	3.08	2.74	8.72	13.54	7.9	2.05	4.31	2.6	7,66	13.61	5.49	2.11
L.S.D. (5%) (20*40 cm)	3.22	2.68	7.41	10.08	5.86	1.93						

Table (5) **Mean values of some growth parameters of *Galega officinalis* under two different spacing as influenced by different fertilizer treatments (26/11/2008)**

	20 cm						40 cm					
Treatment	**Plant height (cm)**	**Sucker No.**	**Fresh weight whole plant (g)**	**Leaves fresh weight (g)**	**Leaves dry weight (g)**	**Fruit wt. (g)**	**Plant height (cm)**	**Sucker No.**	**Fresh weight whole plant (g)**	**Leaves fresh weight (g)**	**Leaves dry weight (g)**	**Fruit wt. (g)**
Control	***50.43***	***46.87***	***599.33***	***399.77***	***101.94***		***43.10***	***23.77***	***292.50***	***201.00***	***51.26***	
Cattle manure	***56.10***	***30.43***	***630.00***	***554.33***	***141.36***		***55.20***	***31.43***	***502.53***	***308.33***	***78.63***	
Rhizobacterin	***53.43***	***24.33***	***540.87***	***330.10***	***84.18***		***42.87***	***22.10***	***557.33***	***385.43***	***98.29***	
Phosphorine	***42.53***	***24.00***	***646.77***	***502.43***	***128.12***		***62.20***	***43.67***	***1260.67***	***925.67***	***236.05***	
Compost	***56.00***	***43.67***	***1011.20***	***668.67***	***170.51***		***50.43***	***37.33***	***753.53***	***535.33***	***136.51***	
Ammonium nitrate 100kg/fed	***50.53***	***42.10***	***700.67***	***520.67***	***132.77***		***61.43***	***25.00***	***1190.10***	***838.00***	***213.69***	
Ammonium nitrate 200 kg/fed	***48.10***	***37.43***	***885.10***	***622.67***	***158.78***		***69.33***	***57.43***	***2252.00***	***1716.87***	***437.80***	
L.S.D. (5%)	3.92	2.94	38.06	37.11	8.75		4.6	3.92	42.61	45.01	8.22	
L.S.D. (5%) (20*40 cm)	2.84	2.19	35.62	32.19	7.04							

Table (6) **Mean values of some growth parameters of *Galega officinalis* under two different spacing as influenced by different fertilizer treatments(12-03-2009)**

	20 cm					40 cm				
Treatment	**Plant height (cm)**	**Sucker No.**	**Fresh weight whole plant (g)**	**Leaves fresh weight (g)**	**Leaves dry weight (g)**	**Plant height (cm)**	**Sucker No.**	**Fresh weight whole plant (g.)**	**Leaves fresh weight (g.)**	**Leaves dry weight (g.)**
Control	***92.20***	***28.10***	***447.20***	***346.20***	***83.09***	***74.43***	***25.77***	***274.77***	***130.43***	***31.30***
Cattle manure	***77.10***	***53.00***	***441.53***	***347.33***	***83.36***	***74.67***	***32.00***	***343.10***	***258.87***	***62.13***
Rhizobacterin	***73.10***	***28.67***	***412.00***	***282.87***	***67.89***	***62.00***	***35.10***	***359.20***	***248.00***	***59.52***
Phosphorine	***93.87***	***26.53***	***294.67***	***202.67***	***48.64***	***63.53***	***34.10***	***309.67***	***186.77***	***44.82***
Compost	***86.10***	***49.33***	***477.33***	***341.87***	***82.05***	***75.67***	***54.87***	***368.43***	***258.33***	***62.00***
Ammonium nitrate 100kg/fed	***101.67***	***44.10***	***409.20***	***349.33***	***83.84***	***76.67***	***33.32***	***416.37***	***316.53***	***75.97***
Ammonium nitrate 200 kg/fed	***78.33***	***24.43***	***364.33***	***249.87***	***59.97***	***95.33***	***45.00***	***389.43***	***264.10***	***63.38***
L.S.D. (5%)	4.23	1.62	12.13	14.3	7.42	5.23	1.84	16.7	12.6	6.88
L.S.D. (5%) (20*40 cm)	4.01	1.24	9.7	10.22	4.18					

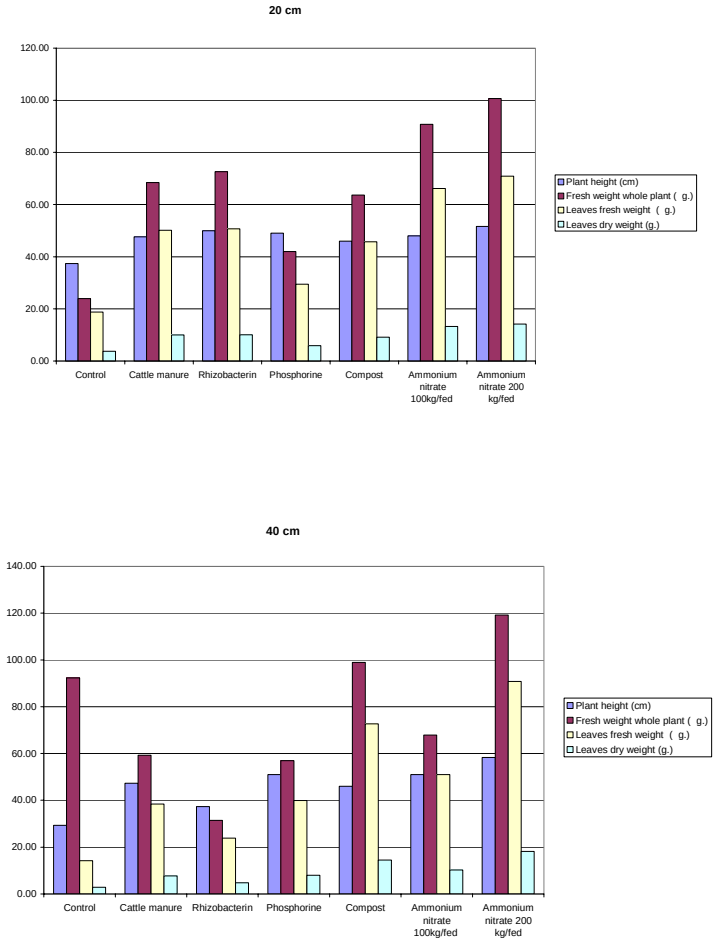


Fig.(1) mean value of some growth parameters of goats rue under two different spacing as influenced by different fertilizer treatments at first sample June 2008.

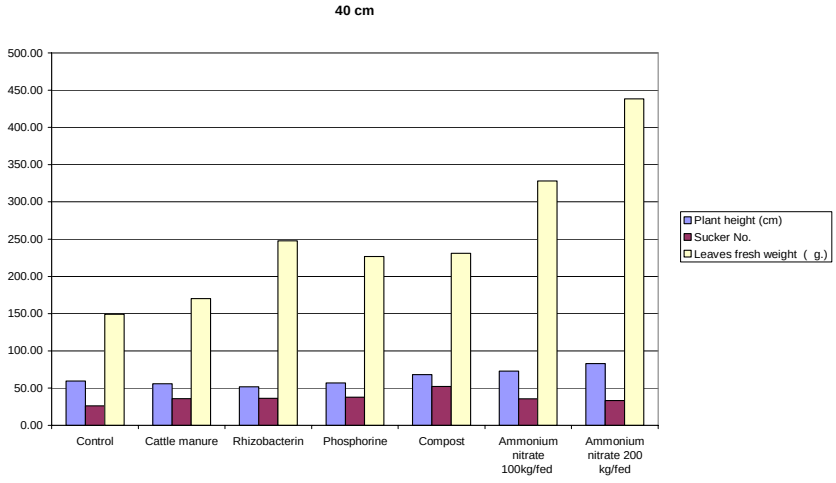


Fig.(2) Mean values of some growth parameters of *Galega officinalis* under two different spacing as influenced by different fertilizer treatments (31-12-2008)

Table (7): R_f values of alkaloid in different extracts and with different solvent systems

	Solvent I	Solvent II	Solvent III	Corresponding to galegine
	R_f values			
Alcohol extract	0.184	0.897	0.50	
Crude alkaloid	0.184	0.890	0.55	
Metformin	0.186	0.893	0.55	

Table (8): Percentage of alkaloid through different plant stages

	Date of cut / Treatments	June	July	Sept.	Nov.	Dec.2008	March 2009
20 cm	Control	7.6	3.0	1.4	1.2	2.2	1.6
	Cattle manure	5.8	3.6	3.2	1.4	3.2	3.6
	Rhizobacterin	5.2	6.8	3.2	1.0	2.6	2.4
	Phosphorine	4.8	0.0	2.8	2.0	2.4	1.6
	Compost	6.2	2.6	2.6	1.8	2.0	2.0
	Amm.Nitrate 100	7.8	3.0	3.0	1.6	2.2	2.4
	Amm.Nitrate 200	7.2	3.6	3.6	1.6	1.0	2.8
	Mean	**6.37**	**3.23**	**2.83**	**1.51**	**2.23**	**2.34**
40 cm	Control	*8.0*	*2.8*	*2.0*	*1.2*	*1.8*	3.6
	Cattle manure	*3.2*	*4.0*	*3.6*	*2.4*	*2.6*	*3.8*
	Rhizobacterin	*7.0*	*3.0*	*4.8*	*1.0*	*2.8*	*2.4*
	Phosphorine	*3.8*	*3.4*	*4.2*	*2.4*	*2.4*	*1.6*
	Compost	*5.0*	*3.0*	*3.2*	*2.4*	*2.2*	*2.2*
	Amm.Nitrate 100	*8.4*	*3.4*	*4.2*	*2.2*	*3.6*	*2.6*
	Amm.Nitrate 200	*6.6*	*3.2*	*4.8*	*1.6*	*2.8*	*2.8*
	Mean	**6.00**	**3.26**	**3.83**	**1.89**	**2.60**	**2.71**

Table (8a): Mean alkaloids content as galegine in leaves of *Galega* plant under different fertilization treatments. (g/ plant)

	Date of cut / Treatments	June	July	Sept.	Nov.	Dec.2008	March 2009
20 cm	Control	0.29	1.00	0.62	1.22	1.77	1.33
	Cattle manure	0.58	1.81	1.71	1.98	2.76	3.00
	Rhizobacterin	0.53	3.48	1.49	0.84	1.74	1.63
	Phosphorine	0.28	0.00	1.95	2.56	1.97	0.78
	Compost	0.57	1.22	1.10	3.07	2.37	1.64
	Amm.Nitrate 100	1.03	1.64	1.54	2.12	2.37	2.01
	Amm.Nitrate 200	1.02	1.26	2.13	2.54	0.56	1.68
	Total	**٤.٣٠**	**١٠.٤١**	**10.54**	**١٤.٣٤**	**١٣.٥٣**	**١٢.٠٧**
40 cm	Control	***0.23***	***0.76***	***0.58***	***0.62***	***0.75***	***1.13***
	Cattle manure	***0.25***	***0.98***	***1.53***	***1.89***	***1.24***	***2.36***
	Rhizobacterin	***0.33***	***0.92***	***2.12***	***0.98***	***1.94***	***1.43***
	Phosphorine	***0.30***	***0.74***	***2.34***	***5.67***	***1.52***	***0.72***
	Compost	***0.73***	***2.12***	***1.52***	***3.28***	***1.42***	***1.36***
	Amm.Nitrate 100	***0.86***	***1.20***	***1.58***	***4.70***	***3.31***	***1.98***
	Amm.Nitrate 200	***1.20***	***1.27***	***3.52***	***7.00***	***3.44***	***1.77***
	Total	**٣.٨٩**	**٨.٠٠**	**١٣.١٩**	**٢٤.١٣**	**١٣.٦٢**	**١٠.٧٥**

Fig.(3) Mean alkaloids content as galegine in leaves of Galega plant under dfferent distance

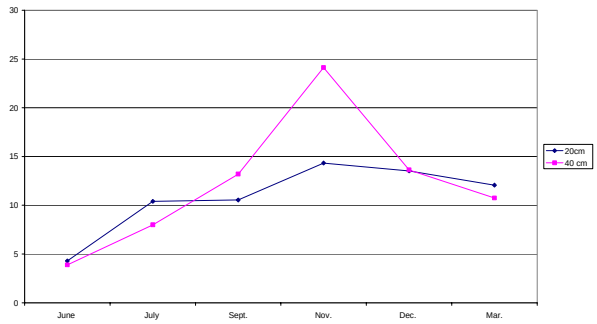
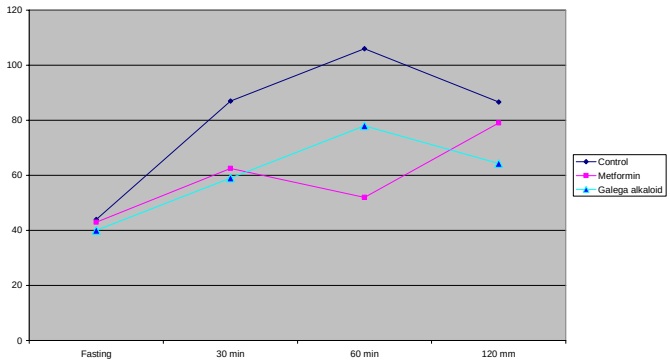


Fig. (4) Effect of metformin and galega total alkaloid on the oral glucose tolerance in rats.



Dealing with the effect of fertilization on the percentage of alkaloid no clear effect was observed due to fertilization. Control plant accumulates.more alkaloid than all the treatments. So, it can be concluded that alkaloid are plant stage dependent and not influenced by fertilization. On the other hand plant growth was affected by different fertilization applied which reflects on the production of alkaloid per plant, represented in Table (8) and Fig (3).

5. Conclusion

Ammonium nitrate in its two rates was the treatment of choice to increase the vegetative parameter studied. Compost or cattle manure came in the next rank. Wider distance of propagation (40 cm) is the most suitable cultivation distance. One major alkloidal spot was detected in crude alkaloid extract and in alchoholic one. Total alkaloid is higher in young leaves then decreased through maturation, when this alkaloid administered to rats it proved to have anti-diabetic activity.

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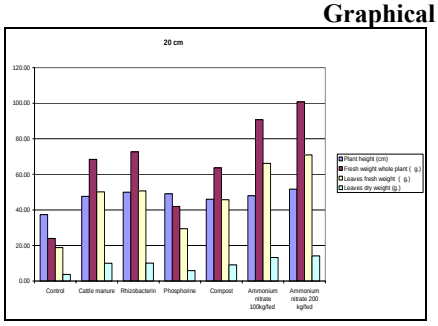
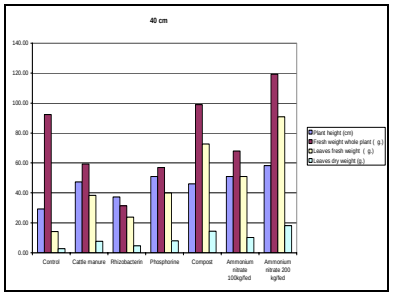
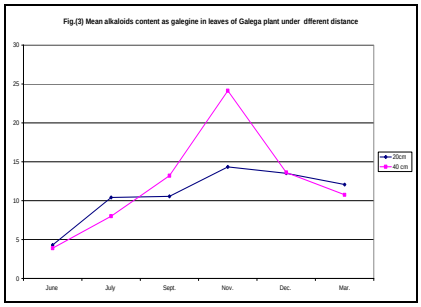
Graphical Abstract







The mineral nitrogen proved to be the effective source followed by compost then cattle manure on growth and yield of leaves, whole plant, flower, and sucker number. Total alkaloid was determined in leaves and it fluctuated with no clear trend, however was higher in sample taken at June.

2/1/2011