International Standard Serial Number (ISSN): 2249-6807

International Journal of Institutional Pharmacy and Life Sciences 2(3): May-June 2012

INTERNATIONAL JOURNAL OF INSTITUTIONAL PHARMACY AND LIFE SCIENCES

Pharmaceutical Sciences **Research Article……!!!**

Received: 04-06-2012; Revised; Accepted: 07-06-2012

BIOCHEMICAL CHANGES DURING GERMINATION OF VIGNA UNGUICULATA EFFECT OF BIO FERTILIZER 06-EM & MA

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Keywords:

Vigna unguiculata, protein, glucose, and anti-inflammatory activity

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ABSTRACT

To study the germination rate of *Vigna unguiculata* under various treatments invoving O6-EM & MA with NPK and Cow dung. The increase in seed weight and shoot length during the germination. To determine the biochemical changes (soluble protein content and glucose content) of *Vigna unguiculata* during germination and its relation to the changes in seed weight and study the anti-inflammatory activity of Calcium alginate.

INTRODUCTION

Germination is a critical stage in the life cycle of weeds, medicinal and crop plants and often controls population dynamics, with major practical implications [1], plant growth regulators such as GA3 (gibberellic acid) hot water treatments have been recommended to break dormancy and enhance germination. Tropical developing countries are facing an increasing demand for protein-rich food due to teeming population, cereal-based diet and scarcity of fertile land [2, 3].

Legumes are an in expensive source of proteins with desirable characteristic such as abundance of carbohydrates, ability to lower the serum cholesterol, high fiber, low fat (except oilseeds),high concentration of polyunsaturated fatty acids and a long shelf life. In addition B complex vitamins, minerals and fiber, legumes are also major sources of proteins and calories [4]. They are known to contain certain bioactive compounds whose beneficial effects need to be explored for exploitation. Seaweed fertilizer was found to be superior to chemical fertilizer because of the high level of organic matter aids in retaining moisture and minerals in the upper soil level available to the roots [5].

MATERIALS METHODS

Collection of seaweeds

Germination studies: The seeds of *Vigna unguiculata* used in this study were collected from the local seed market

Vigna unguiculata

Family Name: papilionaceae

Genus : vigna

Species : unguiculata

Vernacular Name: English: black – eyed Pea, Tamil : thatta payir.

When the seeds were soaked in dilute solution of manure for about 8 to 12 hours, the manure is absorbed by the seeds. The germination and growth of the seeds are measured, compared with other manures and the efficiency of manure in germination and growth promotion of plant is estimated.

Determination of soluble protein by Lowry's method

The method developed by [6] was followed to estimate the amount of protein present in the sample. Proteins react with Folin-Ciocalteau reagent to give a blue colored complex. The intensity of colour depends measured calorimetrically at 640 nm.

Estimation of Glucose by O-Toluidine method

Glucose was estimated by the method of [7]. Glucose when heated with o-toluidine in glacial acetic acid forms N-glycosylamine, which is a bluish green complex. Its color intensity can be measured calorimetrically at 640 nm.

Assay for acute anti- inflammatory activity

Carrageen an-induced Paw Edema Test

Swiss albino mice (20-30 g) were supplied by a local supplier. The animals were maintained under standard laboratory conditions (22-28^{o}C, 60-70 % relative humidity, 12 hrs dark/light cycle) at adthiparasathi college arts and science, kalavai, Vellore (Dt) and fed with standard rat feed (S.K.M. Feeds Ltd., Erode) and sterile water until further use.

Anti-inflammatory activity was measured in mice using Carrageen an-induced paw edema model [8]. Animals were divided into 3 groups of six animals per group.

Group I: Animals were given distilled water control;

Group II (experimental group): Animal was given Ibuprofen (250 mg/kg);

Group III: was given Ibuprofen (250 mg/kg); and (250mg/kg) of Calcium alginate.

After 1 hr of treatment, sub plantar injection of 0.02 ml of 1 % Carrageen a was given into the right hand paw of mice. The degree of edema formation was assayed as increase in paw thickness [9] was measured using screw gauge. Development of edema following the injection of Carrageen and was the index of acute inflammatory changes, which was determined from the differences of the paw thickness measured immediately after Carrageen an injection and then at hourly intervals for up to 5 hrs.

Measurement of seeds height, fresh and dry weight Status

The length of each seedling was measured after 13 days (the seeds had been soaked in fresh water for 5 days prior to the sowing). The fresh weight of each Culm with its leaves was taken, and then the samples were oven-dried at 80°C and the dry weight was taken. To determine relative water content (RWC), four plants from each treatment were randomly selected and the method. Described by Weatherly and Turner was followed [30-31]. About 0.1 g leaf sample was cut into smaller pieces and weighed to determine initial weight (WI). The leaf samples were then floated in freshly de-ionized water for 12 hr. And weighed there after to determine fully turgid weight (Wf). The sample was oven-dried at 80˚C for 3 days and the dry weight was obtained (WD). The relative water content (RWC) was determined using the following formula: (Fig-2)

$$RWC = (WI - WD)\ (Wf - WD)\text{-}1\ 100.$$

Germination studies

Germination Rate of Seeds

Table 1 gives a summary of the 7 treatments under observation, the numbers of *Vigna unguiculata* seeds planted, the numbers of seeds which germinated during the 1-week term of the experiment, and the percentage of seeds germinating. The treatments (at 0.2 % concentration in water) used were:

1. Distilled water (Control)
2. NPK – Chemical fertilizer (CF)
3. Cow dung – Biofertilizer (CD)
4. O6-EM & MA – Liquid Gelly Biofertilizer (G)
5. O6-EM & MA + NPK (G + CF)
6. O6-EM & MA + Cow dung (G + CD)
7. O6-EM & MA + NPK + Cow dung (G + CF + CD)

All treatments gave 95 to 100% germination rate. It is apparent that there were no great differences between different treatments in germination rate. The percentage of germination was well above than that observed in ordinary nursery practice.

Table 1.

Germination rate of *Vigna unguiculata*

Parameter	No. Planted	No. Germinated	% Germination
Control	40	39	97.5
Fertilizer (CF)	40	39	97.5
Cow Dung (CD)	40	40	100
Gel (G)	40	38	95
G + CF	40	39	97.5
G + CD	40	40	100
G + CF + CD	40	39	97.5

Changes in Seed Weight during Germination

During the one week period, seed weight of *Vigna unguiculata* increased by about 51% for control seeds, while it was 72% for seeds treated with NPK, 173% for seeds treated with cow dung and 86% for seeds treated with the gelly liquid manure O6-EM & MA. When the seeds

were treated with O6-EM & MA + NPK, the growth was 134%, while it was 57% for both O6-EM & MA + cow dung and O6-EM & MA + NPK + cow dung (Fig- 1). Thus the growth stimulation of O6-EM & MA was lower than that of cow dung and higher than that of NPK. The O6-EM & MA and NPK together showed higher stimulation, suggesting a synergistic relationship between them.

Fig: 1

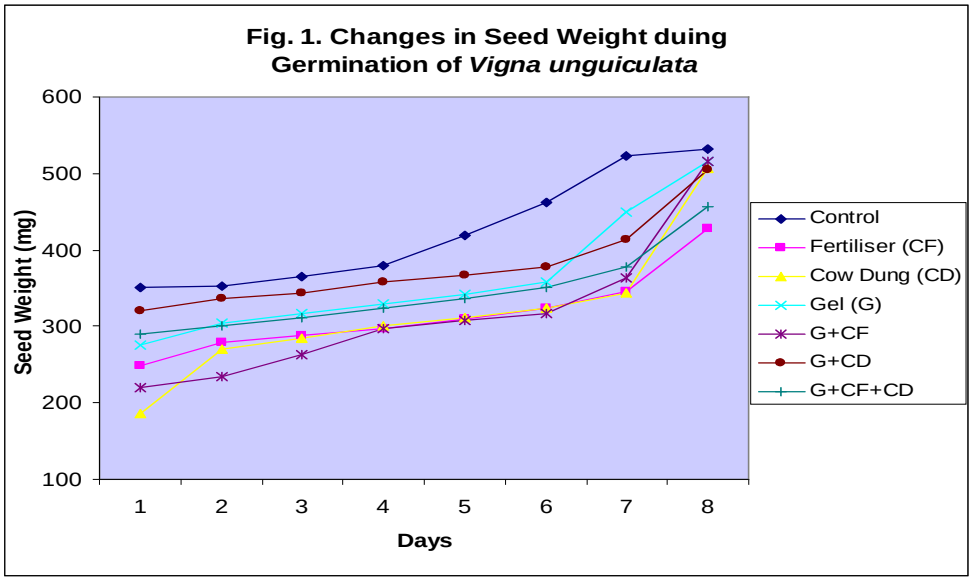


Fig: 2

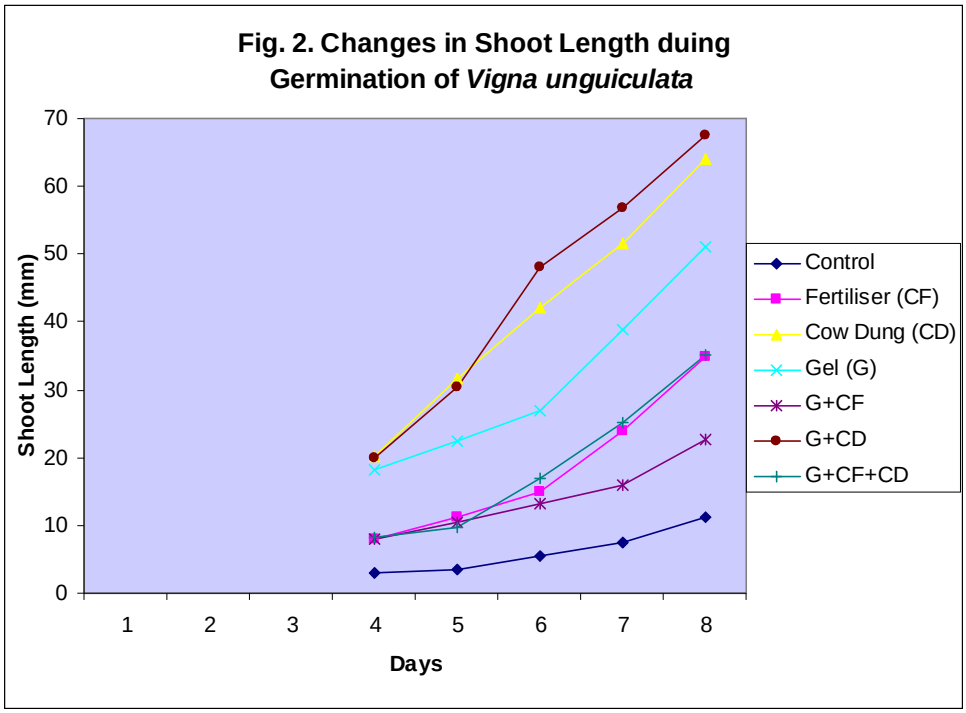


Fig: 3

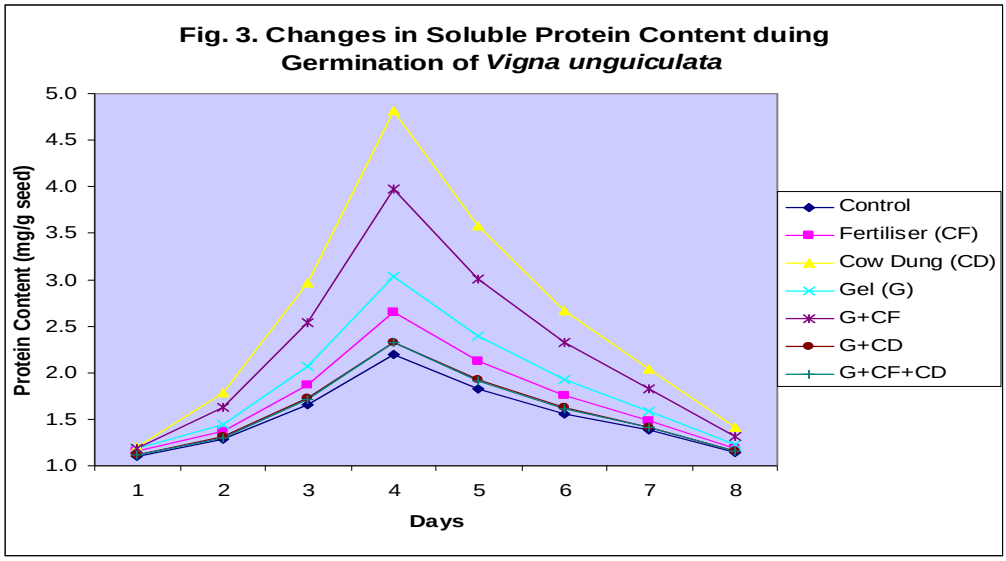


Fig-3 shows the evolution of soluble protein content during the process of germination of *Vigna unguiculata.* In general, germinating slightly increased protein content from 1 to 4 days. This correlates with the results reported by [10, 11]. All the treatments showed similar pattern with difference only in the magnitude of increase in protein content. Maximum protein content was found in the treatment of Cow dung, followed by O6-EM & MA + NPK, O6-EM & MA, O6-EM & MA + Cow dung, O6-EM & MA + NPK + Cow dung, and NPK, with all of them showing an increased level than the Control. This closely parallels the pattern found in the increase of seed weight with these treatments.

Fig: 4

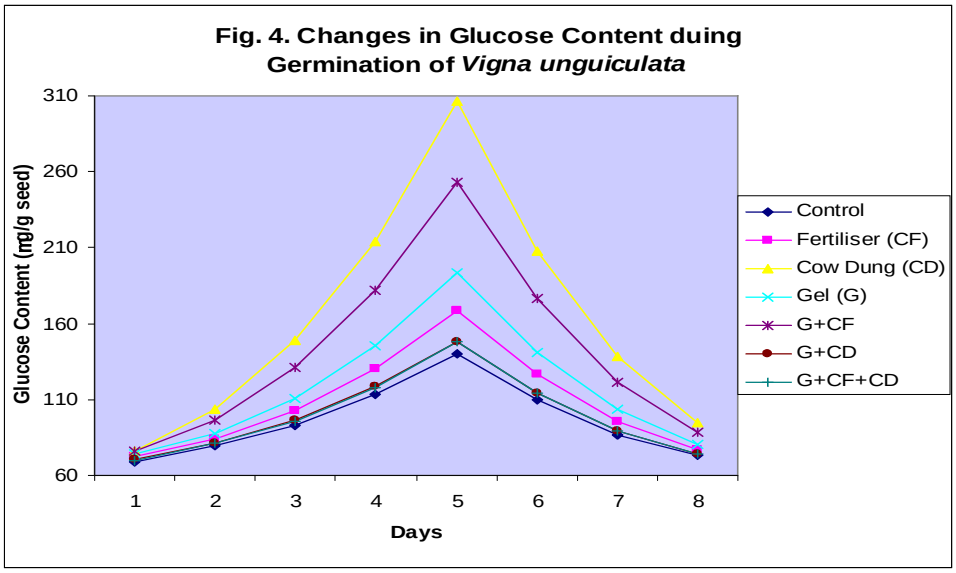


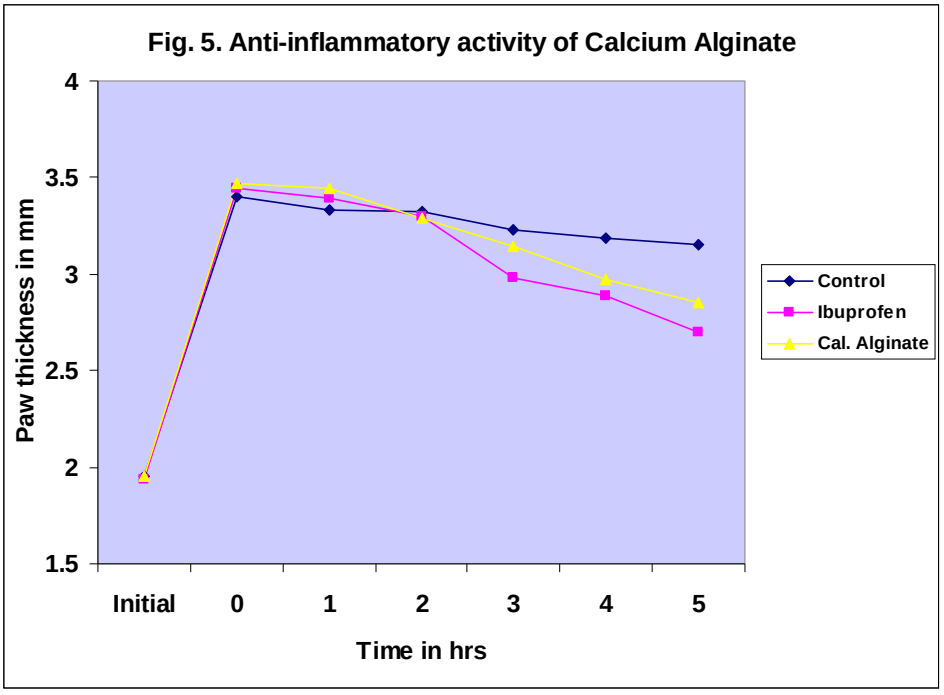
Fig-4 show the evolution of glucose content during the process of germination of *Vigna unguiculata*. In general, germination slightly increased glucose content from 1 to 3 days. This correlates with the results reported by [12]. All the treatments showed similar pattern with differing only in the magnitude of increase in glucose content. Maximum glucose content was found in the treatment of Cow dung, followed by O6-EM & MA + NPK, O6-EM & MA, O6-EM & MA + Cow dung, O6-EM & MA + NPK + Cow dung, and NPK, with all of them showing an increased level than the Control. This closely parallels the pattern found in the increase of seed weight and soluble protein content with these treatments.

Acute anti- inflammatory activity

Carrageen an-induced acute paw edema inflammation is one of the suitable test procedures to screen anti-inflammatory activity. The development of carrageen an-induced edema is biphasic: the first phase is attributed to the release of prostaglandins. Carrageen an induces paw edema by inducing protein-rich exudates containing large number of neutrophils.

The acute inflammatory activity of Calcium alginate was tested by carrageen an-induced paw edema model [8]. The Calcium alginate showed lower anti-inflammatory activity but when compared to the ibuprofen activity was slightly reduced (Fig-5).

Fig: 5



CONCLUSION

In this conclusion, attempts were made to compare a new biofertilizer O6-EM & MA with the chemical fertilizer NPK and the traditional biofertilizer Cow dung in the germination of *Vigna unguiculata* seeds. This study also tried to demonstrate the possible relationship between soluble protein content and glucose content of seeds with the increase in seed weight during the process of germination.

The possible level and role of plant hormones such as auxins and cytokinins in this process has to be studied for further understanding of this work. As this product is formed as byproduct during the production of Calcium alginate, the anti-inflammatory activity of Calcium alginate was also studied and found to have a slight anti-inflammatory activity.

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