International Journal of Research Publication and Reviews

Journal homepage: www.ijrpr.com ISSN 2582-7421

MODIFICATION IN DNA BASEPAIRS OF SEEDLINGS (VIGNA RADIATA) DUE TO USAGE OF FERTILIZERS

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ABSTRACT

The commonly used fertilizers are known to cause severe nucleic acid damages in agricultural crops. The present study was conducted to investigate the influence of organics as well as chemical fertilizers on the growth and yield of vigna radiate (green gram or mung bean) and to assess the fertilizers induced DNA damages in green gram. This experiment was conducted for 15 days by treating the seedlings with different fertilizers. Treatment inputs were control (without adding fertilizers) (G1), the mung bean (Vigna radiata) vermicompost (G2), Urea (G3), mixture of ammonium sulphate +phosphate +nitrate and neem cake (G4). All these were added in same quantity, seedlings were allowed, and the seedling pattern was observed for 15 days. Then DNA was extracted from leaflets and separation of DNA bands were visualized using agarose gel electrophoresis to assess the influence of fertilizers in DNA changes. The smear of DNA bands was mostly visualized in G3 and G4 when compared with G1 and G2 indicating vermicomposting was the best treatment for the production of green gram in high yield without effecting its DNA.

Keywords: *Fertilizers, Green gram (vigna radiata), vermicompost, yield, DNA damage, Agaroe gel.*

1. INTRODUCTION

Several types of natural and industrial fertilizers are being currently used in agricultural practices worldwide. Typically, any type of fertilizer is known to contain a minimum of 5% of primary nutrients as nitrogen (n), potassium(k), phosphorus (p) and additional N, P, K, etc. as major and Zn, Mn, Fe, etc. as minor elements. This composition includes minerals and natural manures and furthermore soil conditioners, for example, lime and gypsum, which might advance plant development by expanding the accessibility of supplements that are as of now in the soil or by changing the soil's actual composition and physical structure. Liquid fertilizers are applied with artificial water system or for application straight in soil. Simplicity of taking care of, less work necessity and plausibility of blending in with herbicides have made the fluid fertilizers more adequate to agricultural crops.

Based on complexity of fertilizers supplies just a single essential plant supplement, specifically nitrogen or phosphorus or potassium. For example, urea, ammonium sulfate, potassium chloride and potassium sulfate supplies a few essential plant supplements of which two essential supplements are in chemical mixtures. These fertilizers are available to farmers as a granule. In agricultural practices, the soil is considered as the essential substrate for plant growth. In addition, it also forms suitable environment for growth of soil organisms and as such a recycling system in ecology. Therefore, the soil as an environment of soil organic entities, is a supplement reusing framework and gives numerous other biological growth. The excessive use of industrial fertilizers can prompt soil fermentation and soil crust by decreasing organic matters, humus content, symbiotic microorganisms, hindering plant development, can change the soil pH, increase the pests and even add to the arrival of greenhouse gases. The soil acidity reduces phosphate consumption of the crops, expands the harmful particle fixation in the soil, and diminishes crop development. The consumption of humus in the soil decreases its capacity to store supplements. Ozone depleting substances obtained from unutilized nitrogen fertilizers can potentially affect the environment. Nitrogen applied to fields in huge sums obliterates the harmony between the three macronutrients, N, P and K over the long haul, which would bring about absence of micronutrients; it additionally harms dirt, bringing about diminished crop yields.

Sandy soils are substantially more inclined to soil fermentation than are mud soils. Mud soils have a capacity to buffer the impacts of overabundance synthetic preparation. Frequent uses of manures might bring about accumulation of toxic metals like arsenic, cadmium, and uranium in the soil. These metals not only cause toxicity to the soil but also get accumulated in food grains and all agricultural yields. For instance, Fertilizers like triple superphosphate has minor components like cadmium and arsenic that gets accumulated in plant and through natural food chain orders reach to human that might cause toxic issues. The after effects of such fertilizers on soil are extraordinary and irreversible. Manure application without the utilizing soil-testing proposal can prompt ramifications, for example, soil contamination, depletion of nutrient supplements, loss of soil structure, expanding soil mass thickness. Fertilizers, more than the suggested concentrations cause unwanted accumulations and grouping of mineral salts of fertilizers, which prompts compaction layers and soil degradation in the long haul.

Fig 1 : The mung bean (Vigna radiata), alternatively known as the green gram, maash, moong, is a plant species in the legume family

The mung bean (Vigna radiata), on the other hand known as the green gram is a plant variety in the vegetable family (Fig 1). It is chiefly developed in East and southeast Asia and India. It is utilized as a fixing in both flavorful and sweet dishes. In earlier literatures, studies in agriculture fields were conducted to evaluate the effect of natural fertiizers on development and yield of green gram crops. The most extreme yield of Green gram (vigna radiata L) crop was subjected to vermicompost therapy following 60^{th} day compared with other fertilizers demonstrating that vermicompost was the ideal natural blend of fertilizer treatment for maximum yield of mung beans. Inorganic manure was related with decrease in a few soil properties and harvest yields after some time. It was observed that use of natural fertilizers has beneficial outcomes in maintaining the soil properties. A few studies investigated to concentrate on the development and physiology of peanuts on various portions of cow excrement and NPK 16 composts. Such studies indicated that there was relationship among fertilizers and NPK 16 portions to add up to chlorophyll content of leaves. Independently, cow excrement and NPK 16 affected in plant tallness, ANR and absolute chlorophyll contents of leaves.

In another study used the seeds of mung bean type Vamban2 were subjected with various portion of physical (Gamma rays) and mutagens like Ethylmethane sulphonate to cause DNA damage. The resultant reactions were considered in M1 and M 2 stages and range of chlorophyll transformation were assessed. Different chlorophyll types were noticed, like Albina, xantha, chlorina and viridis. In further interpretation of study, it was found that the transformation recurrence increased with raise in the dosage of mutagen. As a rule, the chlorophyll freak was higher in EMS than the gamma beams treated plants. In this paper we evaluated the effect of vermicomposting treatment in improving the crop production of green crop by observing the experimental condition for 15 day for seedling growth, morphometric analysis and nucleic acid damages by agarose gel electrophoresis.

2. MATERIALS AND METHODS

Seedlings (sprouts) of vigna radiate (green gram). We used green gram because it grows very faster when compared to other seedlings. The seeds of green gram were taken and soaked for 4-6hrs and then we tied them in a cloth by draining of water and left over for a night to become sprouts. These sprouts were taken in 4 petriplates which are layered with cotton and filter paper and sprinkle water on the layers, to stick the layer to the plates. In each petriplate, place 5 to 7 seedlings and add little amount of water to all the petriplates which are named as G1, G2, G3 and G4. Take the fertilizers (vermicompost, urea & mixture of Ammonium nitrate, sulphate and phosphate +neem cake) and dilute them (1g/100 ml). Then add 10-15ml of fertilizers in each of petriplate. And those seedlings were allowed to grow for 12-15 day. After 15 days, small leaflets were observed in each petriplate. The DNA extraction was carried out using those leaflets followed by agarose gel electrophoresis.

Agarose gel electrophoresis:

Agarose gel electrophoresis is the most common method used in analysis and observing the nucleic acid separation (DNA or RNA) based on molecular size. This was carried out through movement of negatively charged DNA or RNA in agarose gel electrophoresis with an electric field (electrophoresis). Smaller molecules mover faster and separate longer than heavier molecules. Nucleic acid separation is usually carried for analytical purposes. DNA can be electrophoresed through the gel prepared by melting and re-gelling agarose. In agarose gel electrophoresis, D-galactose as a copolymer enable formation of pore size depending on percentage of agarose used. The movement nucleic acids in agarose was influenced by agarose concentration. Increasing the agarose concentration of the gel reduces the migration speed and enables separation of smaller DNA molecules. The separation of DNA under electrophoresis is dependent on voltage applied which enable size based movement There are several DNA detection methods are used to visualize the nucleic acids in agarose gel electrophoresis, such as ethidium bromide which can fluorescence with UV light illumination. In normal light illumination fluorescent bands are not visible.

In electrophoresis, xylene cyanol and bromophenol are mixed in buffers as they migrate in electricity field along with DNA fragments, which are 5000bp and 300bp in lengths. Generally, agarose gels could be made in the range of 0.7% and 2% using electrophoresis buffer. It is common practice to use Tris/acetate EDTA (TAE) and a few other buffers has lower buffering capacity but results in better images of larger DNA. Another buffer made with Tris/borate EDTA has a greater buffering capacity producing better resolution of nucleic acid images. It was found that TBE buffer produces improved resolution of 0.1 to 3 kb fragments; However, Tris-Acetate-EDTA buffer show improved images with larger than 4 kb fragments. Agarose being made of polysaccharide are obtained from certain seaweed having a polymer unit of agarobiose made up of D-galactose and 3, 6-anhydro-L-galactopyranose. The more the agarose percentage used the “stiffer” the gel becomes.

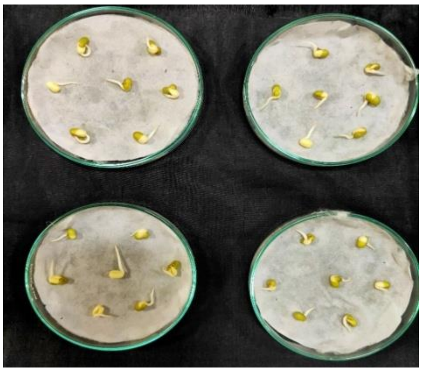

Fig 2: The fertilizers treated seedlings were allowed to grow for 15 days and results observed as shown in petridishes.

Day	Observations
1st day	No growth in the seedlings
3rd day	Small growth of shoot in each group
5th day	There is a little bit increase of shoot growth in G1,G2 and no change in G3 and G4
7th day	Small leaflets are grown in G1,G2 and increase in shoot of G3,G4
9th day	There is an increase in shoot length and leaflets were raised in G3 ,G4
11th day	More increase in growth of shoot in all the plants of all groups
13th day	Increase in shoot length in all groups and other leaflets were raised from G1,G2
15th day	All the leaflets in all the groups were grown completely.

Table 1 : The observation of changes in seedlings growth pattern treated with fertilizers upto 15 days

S.No	Shoot length	Root length	Leaf count	Weight	Total length of the plant
1st plant	2.3cm	1.5cm	2	0.098g	3.8cm
2nd plant	3.2cm	1cm	2	0.082g	4.2cm
3rd plant	2.5cm	1.2cm	2	0.100g	3.7cm
4th plant	5.8cm	1.8cm	2	0.108g	7.6cm
5th plant	7.2cm	2.4cm	2	0.116g	9.6cm
6th plant	10.3cm	1cm	2	0.157g	11.3cm
7th plant	1.2cm	2cm	2	0.119g	13.2cm

Table 2 : Morphological analysis of fertilizers treated seedlings.

DNA ladder : Lane 1 2 3 4

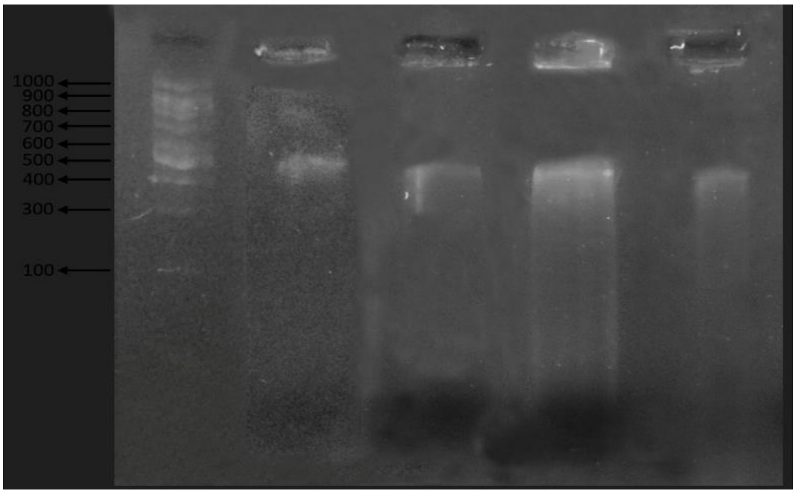

Fig 3 : Agarose gel electrophoresis of extracted DNA samples. Lane 1: Control (no fertilizer), Lane 2: Vermicompost extract, Lane 3: Urea treated extract and Lane 4: Mixture of ammonium nitrate, suphate and Phosphate with neem cake. That smear appearance of the DNA bands was due to the effect of fertilizers on the plants.

3. RESULTS AND DISCUSSION

The seedlings were grown by arranging a cotton bed and filter paper and adding water and fertilizers in required amount and allowed to grow (Figure 2). The results of observation of seedlings growth were shown in Table 1. After noting the morphological analysis by taking measurements of shoot length, root length, leaf count, weight and total length (Table 2), then 2/3rd of leaves was cut into pieces in all the groups and the total DNA was isolated from leaves separately in each group and then followed by agarose gel electrophoresis. The DNA isolated from the plants was inserted in wells and then run the gel for 30-40 minutes. The bands of DNA was visualized under UV light as shown in Figure 3. The bands of the DNA indicated a DNA smear.

4. CONCLUSION

Our observations Indicated that the use of Vermicompost in agriculture production of Green gram is more useful for better yield. Even though fertilizers are useful in promoting the growth, they have many adverse effects which are damaging the soil, causing pollution to water and also the genetic material of the plant and its productivity, the observation of smear bands of DNA in agarose gel electrophoresis was due to the action of fertilizers on the plant DNA. The smear appearance of DNA bands is more in 3rd and 4th well is more when compared to 1st and 2nd wells. That smear appearance is due to action of chemical fertilizers on the plant genetic material. Our observation suggested to reduce the usage of chemical fertilizers and improve the usage of biofertilzers as they are eco-friendly to nature minimizing plant DNA damages.

Acknowledgement: The authors thank Bharath Institute of Science and Technology, Chennai, India, for their encouragement and support for successfully completing above research work.

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