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Fabrication of Nano Structured Slow Release Fertilizer System and its Influence on Germination And Biochemical Characteristics of Vigna Raidata

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Abstract : Increased application of fertilizer to land pollutes the environment and sustainable productivity of land is hampered by the loss of Plant Growth Promoting Rhizobacteria. So an attempt was made to fabricate nano structured slow release fertilizer system associated with neem cake and Plant Growth Promoting Rhizobacteria. Nano structured fertilizer was prepared by ultra sonication and slow release fertilizer system was fabricated by a composite of neem cake, plant growth promoting rhizobacteria and nano NPK fertilizer. The influence of slow release fertilizer system on germination, mean germination time, seed vigour index, amylase, protease, total carbohydrates and total proteins of *Vigna radiata* was evaluated in pot study. Treatments for germination study included water control (T1), neem cake control (T2), NPK control (T3), phosphate solubilizing bacteria control (T4), potash solubilizing bacteria control (T5), nano NPK (T6), neem cake and nano NPK (T7), neem cake and phosphate solubilizing bacteria (T8), neem cake and potash solubilizing bacteria (T9) and slow release fertilizer system (T10). Size of nano fertilizer ranged from 64 to 73 nm. Germination was 85% in T1, 87 % in T2, 82% in T3, 89% in T4,T5 and T6,91% in T7, 86% in T8 and T9. 100% of germination was observed in slow release fertilizer system. Mean Germination Time in T1 was 42.5 and it was remarkably increased to 100 in T10. Seed vigour index was 423 in T1 and 1625 in T10. Slow release fertilizer system has accelerated the enzyme activity during germination and it was responsible for the observed increase in seed vigour index.
Keywords : Slow release fertilizer system, nano fertilizer, mean germination time, seed vigour index and *Vigna radiata.*

Introduction

World population is expected to increase to 2.3 billion people by 2050[1]. To meet the socio-economic demands of exploding population, urbanization and industrialization at the cost of crop land becomes inevitable. Increase in food production / hectare of land is not evolving at the pace of population increase and the planet has virtually no more arable land or fresh water to spare. To feed the ever increasing population and to avoid food demand, agricultural productivity has to be increased by 60 % in 2050. Nitrogen, phosphorus and potassium (N, P and K) are the major plant nutrients essential for foliar formation, root growth and enlargement, phytohormone biosynthesis, biosynthesis of proteins and nucleic acids, flower and fruit development. By repeated cultivation of crops, soil is depleted of essential nutrients and soil is fortified by the addition of commercial NPK fertilizers.Use of fertilizers is justified to increase the crop productivity and to feed the growing population[2]. Approximately 40-75% of applied fertilizers is lost into the environment by leaching and pollutes land and ground water. It causes wastage of fertilizers and also economic loss[3,4]. Pollution due to nitrates from nitrogen fertilizers cause methemaglobinemia in infants,formation of carcinogenic nitrosamine in human stomach, hypertension, eutrophication, formation of algal bloom and oxygen depletion in aquatic environment[5]. High concentration of nitrate in drinking cause nitrate toxicosis. Zhang *et al*[6] has reported the positive correlation between oesophageal cancer and nitrogen compounds originating from fertilizer.

Even though organic fertilizers contain a spectrum of plant nutrients, their nutrient ratio is not guaranteed. Nutrients are released from them according to the conditions prevailing in the soil. Slow Release Fertilizers System is a viable alternative to increase crop productivity without causing environmental pollution.

Some currently available slow release fertilizers are sulphur coated urea, resin coated urea, isobutylidene diurea (IBDU), methylene urea and urea formaldehyde. In most cases of existing slow release fertilizer systems, they burst out all the nutrients. Sulphur husks remain in land after the release of urea in sulphur coated formulations. Major disadvantage of polymer coated urea is the high cost than other fertilizers[7]. Coated fertilizers are cracked during transportation and abrasion causes damage in prills and quick release of nutrients. Nanotechnology has a great impact on all branches of Science and Engineering in every walk of life. The term "Nanotechnology" was first coined by Norio Taniguchi in 1974 which is also called as Second Green Revolution[8]. Fertilizers in nano dimension can be efficiently utilized by plants whose pore size is also at nano scale[9]. So present work was aimed to use neem cake (NC) as an organic carrier to be a vehicle for insoluble chemical fertilizers at nano scale and the respective nutrient solubilizing plant growth promoting rhizobacteria (PGPR).

Materials and Methods

Fabrication and characterization of SRFS

SRFS was formulated by an optimum combination of NC, insoluble form of nano fertilizer (NNPK) and the respective fertilizer solubilising PGPR. 100 mg of urea, 50mg of rock phosphate and 50mg of rock potash were mixed in 5 ml of NC oil. The oil suspension was sonicated for 30 minutes at 0.5 cycles at 80 amplitude. The sonicated sample was mixed with 100g of NC. *Pseudomonas* fluorescence and potash solubilising bacteria were added at a density of $2x10^8$ CFU/g of NC for solubilising the insoluble fertilizers. To the powdered SRFS, 0.2% jaggery and sodium alginate were added and pelletized in Sun enterprises, Virudhunagar, Tamilnadu**.** Bacteria, for the fabrication of SRFS and seeds of *Vigna radiata* variety KM2 were purchased from Tamilnadu Agriculture University, Madurai, Tamilnadu, India.Pellatized SRFS was characterized by FESEM. The influence of slow release fertilizer system on germination, mean germination time, seed vigour index, amylase, protease, total carbohydrates and total proteins of *Vigna radiata* was evaluated in pot study arranged in randomised block design. Treatments for germination study included water control (T1), neem cake control-NC (T2), NPK control (T3), phosphate solubilising bacteria control-PSB (T4), potash solubilizing bacteria control -KSB (T5), nano NPK-NNPK (T6), neem cake and nano NPK-NCNNPK (T7), neem cake and phosphate solubilizing bacteria-NCPSB (T8), neem cake and potash solubilizing bacteria-NCKSB (T9) and slow release fertilizer system-SRFS (T10).Germination percentage was evaluated prescribed by ISTA[10], Mean germination time by Ellis and Roberts[11], Seed Vigour Index (SVI) by Abdul baki and Anderson[12], Amylase activity by Miller[13], protease assay by Folin and Ciocalteau[14], Protein by Lowry *et al*[15] and total carbohydrates by Hedge and Hofreiter[16]. All results were the mean of three replicates and graphs were drawn with error bars indicating standard deviation.

Results and Discussion

This study was aimed to formulate organic SRFS for *Vigna radiata*. NC was used as a carrier for the fabrication of SRFS considering its natural biopesticidal activity and multiple ecofriendly attributes. SRFS before and after pelletization and its FESEM image is represented in Figure 1. FESEM shows the multi layered arrangement of NC and the distribution of nanofertilizer. Many pores were also seen in the neem cake layers. Particle size of materials can be reduced by ball milling, high shear mixing and ultrasonication. Ball milling product always contains contaminants from the milling medium. Morphology of nano materials will be heterogenous. Size reduction by ultra sonication is efficient than other two methods. It reduces size of particles by collapsing the solvent. Particle size can be tailored by varying the concentration of the solution, amplitude and the number of cycles[17]. Reduction of particle size and increasing their bioavailability by ultrasonication was also reported by Hongxing Wang *et.al*[18].

Powdered SRFS **Pelletized SRFS** **FESEM image of SRFS**

Figure 1. SRFS

Influence of SRFS on Germination of *Vigna radiata*

Influence of SRFS on Germination of *Vigna radiata* is shown in Figure 2. It was 85% in T1, 87 % in T2, 82% in T3, 89% in T4,T5 and T6,91% in T7, 86% in T8 and T9. 100% of germination was observed in SRFS. It is due to the fact that it optimizes all possible organic and inorganic sources of plant nutrients.

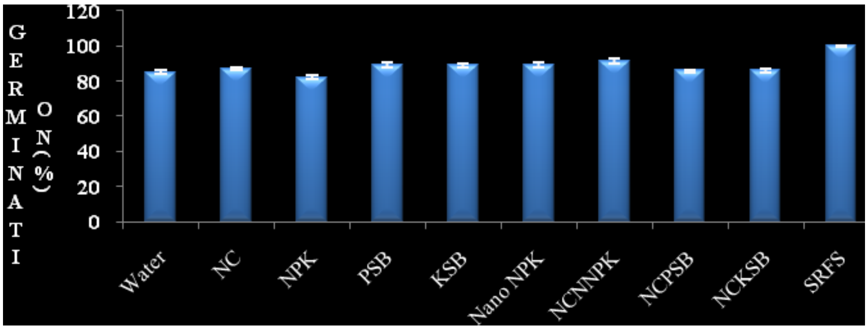

Figure 2. Influence of SRFS on Germination of *Vigna radiata*

NC has dual function in agriculture, functioning as a fertilizer as well as a biopesticide. It serves as a soil conditioner. NC influences seed germination due to the presence of nitrogen, phosphorous and potassium[19]. The influence of SRFS on Mean Germination Time is represented in Figure 3. MGT was 40 in water control and 100 in SRFS. Similarly significant difference in the seed vigour index was observed among treatments. SVI was 226 in control and 629 in SRFS and it is depicted in Figure 4. NC exerts beneficial effect on germination due to its ability to supply the basic nutrients essential for germination. The comparatively reduced rate of germination , MGT and SVI by NPK treatment might be due to the acidity generated by NPK as well as by the formation of ammonia formed by the degradation of urea. This effect is less when urea was complexed with NC.

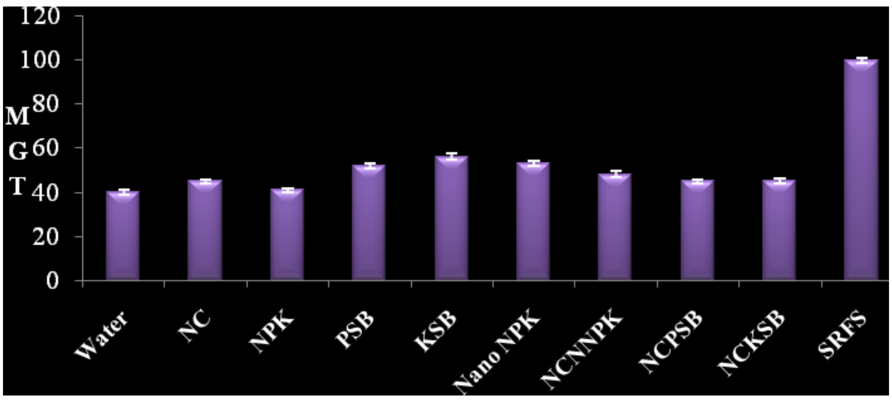

Figure 3. Influence of SRFS on Mean Germination Time of *Vigna radiata*

The influence of SRFS on SVI is represented in Figure 4. SVI was 45 in NC, 41 in NPK and 53 in Nano NPK and 100 in SRFS. The observed difference in SVI is due to the presence of urease inhibitor in

NC.Further in SRFS, the fertilizers were released slowly conducive for germination without causing any inhibitory effect on germination. Influence of fertilizer on germination of vegetables was also studied by Ramteke and Shirgave[20].

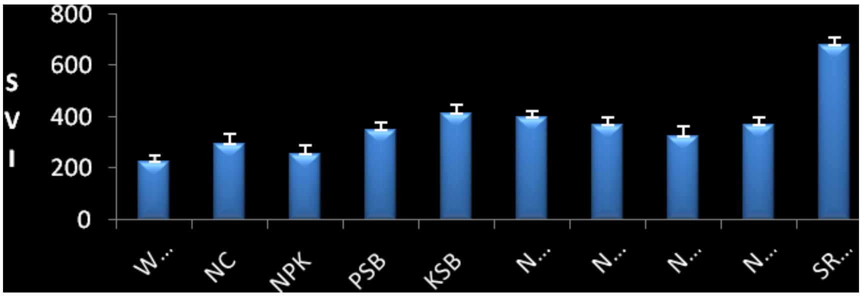

Figure 4. Influence of SRFS on SVI of *Vigna radiata*

PGPR are a diverse group of microbes associated with the rhizosphere soil that improve plant growth both directly and indirectly. In the present study both PSB and KSB had a very good positive influence on germination characteristics of *Vigna radiata.* MGT was 52 in PSB and 56 in KSB. They exerted their influence by facilitating the availability of readily utilizable plant nutrients for germination. Influence of PGPR on germination of chickpea was also studied by Janardan Yadav[21].In SRFS the indirect effect of PGPR in enhancing and stimulating germination might be due to its deleterious effects on phytopathogens. They should have induced germination by synthesizing auxin, gibberellins and cytokinin which aided cell elongation and division[22,23]. Hence root and shoot growth are more in SRFS leading to a remarkable difference in SVI.

Influence of SRFS on Enzyme activity of *Vigna radiata*

The influence of SRS on specific activity of amylase is represented in Figure 5. Amylase specific activity was 3.0 µg/g ,5.29 µg/g ,2.65,4.67,5.39,5.45,7.15,7.92,7and 10µg/g, water control, NC, NPK, PSB, KSB, NNPK, NCNPK,NCPSB, NCKSB and SRFS on 3rd day after germination. It is used to degrade insoluble starch into soluble glucose which is easily utilized by plants and the plant growth was increased. Stimulatory influence of nano gel on amylase activity was reported by Umarani and Mala[24].

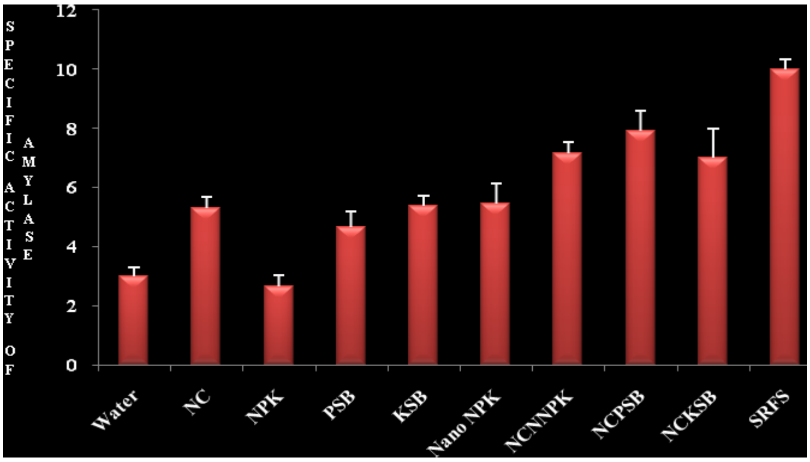

Figure 5. Influence of SRFS on Amylase activity of *Vigna radiata*

Specific activity was 2.65 µg/g in NPK and 10 µg/g in SRFS. This remarkable difference is attributed to many reasons. One fact with fertilizer is that they are released slowly in proportion to their solubilisation by PGPR. Stimulatory effect of fertilizers on enzyme activity was discussed by Černý *et al*[25],.Fertilizers generally induces enzyme activity based on the stage of plant growth and the concentration of the fertilizer In addition, PGPR also increased enzyme activity indirectly by facilitating the availability of nutrients at the vicinity of roots.

The influence of SRS on specific activity of protease is represented in Figure 6. Specific activity of protease was 6.0 µg/g, 8.56 µg/g and 11 µg/g in control, NC and SRFS. It is used to degrade proteins into amino acids for the growth of seedlings. Leguminous plants are known for their riches source of proteins as reserve storage of nutrients. During germination the reserve proteins are hydrolysed by proteases to simpler amino acids essential for the seeds to germinate.

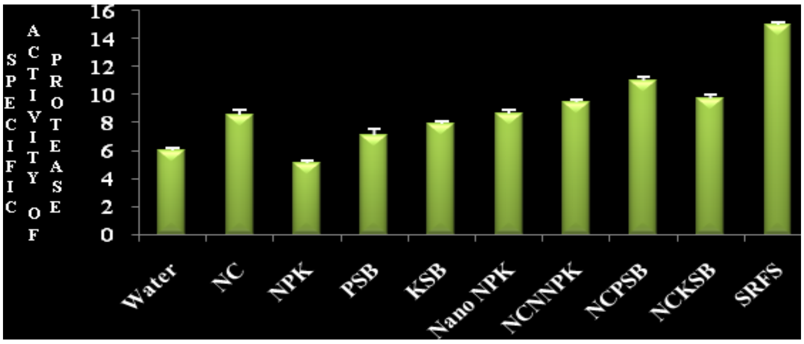

Figure 6. Influence of SRFS on Protease activity of *Vigna radiata*

SRFS treated seeds showed higher activity than all other treatments. This might be due to the optimum absorption of water by seeds. Imbibition plays a key role in activation of enzymes. In Nano NPK the specific activity was 8.65µg/g and in NCNNPK it was 9.45 µg/g. High concentrations of fertilizers around the seeds pose an osmotic pressure and interferes with imbibitions. So NNPK treated seeds had comparatively less activity. Where as in NCNNPK the fertilizers could have been complexed with all other constituents in NC. They only facilitated the slow release of all fertilizers along with PGPR. This is confirmed by the higher most activity recorded in SRFS.

Influence of SRFS on Biomolecules

Proteins are one of the major reserve forms of nutrient in seeds. The influence of SRS on total proteins is represented in Figure 8. The concentration of total protein was 3.0 mg/g, 2mg/g and 0.58 mg/g in control, NC and SRFS.It is used to degrade proteins into amino acids for the growth of seedlings. During germination by the activity of water protease are activated to mobilize the nutrients for the growth of seedlings. So inpart greater the activity of protease greater will be the mobilization and lesser will be the concentration of proteins in the seeds during germination. Free amino acids formed by the action of proteases are transported to the embryonic axis to support growth and as an energy source[26-28]. Less concentration of proteins in SRFS when compared to all treatments was due to the increased activity of protease. Coordinated changes in protein during germination were discussed by Kesari and Rangan[29].

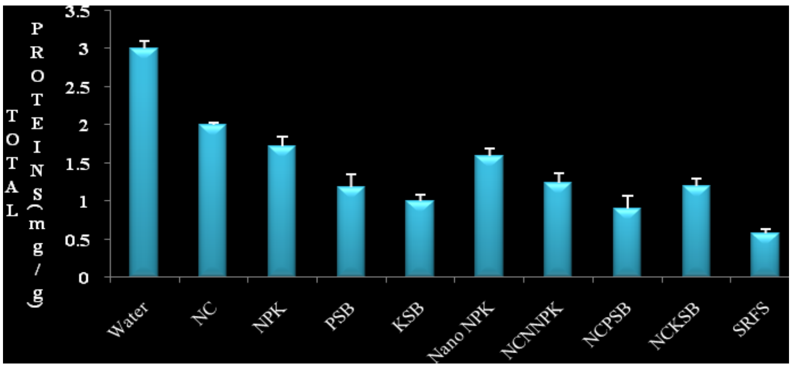

Figure 7. Influence of SRFS on Total Proteins of *Vigna radiata*

Next to proteins, carbohydrates are the major reserve of biomolecule in *Vigna radiate*. Metabolic changes in the nutrient reserve during germination and early growth of seedlings were studied by Černý *et.al*[25].The influence of SRS on total carbohydrates is represented in Figure 8. The concentration of total carbohydrates was 1.25 mg/g ,0.91mg/g and 0.38 mg/g in control, NC and SRFS. Starch is the form of sugar stored in endosperm. Hellmann *et al*[30] reported that starch was most abundant carbohydrate reserve, representing 30-40% of the seed dry weight in *Caesalpinia echinata* seeds. During germination and early seedling growth, starch is hydrolysed to form simple soluble sugars and are mobilized from the endosperm to the embryo for the growth and metabolic energy demands. Hence the concentration of total sugars decrease in proportion to mobilization. Greater mobilization was observed in SRFS (0.38 mg/g) followed by NCPSB(0.47 mg/g) and NCKSB(0.52 mg/g). Changes in carbohydrates during germination was studied by Zanotti Rafael Fonsêca *et.al*.[31].

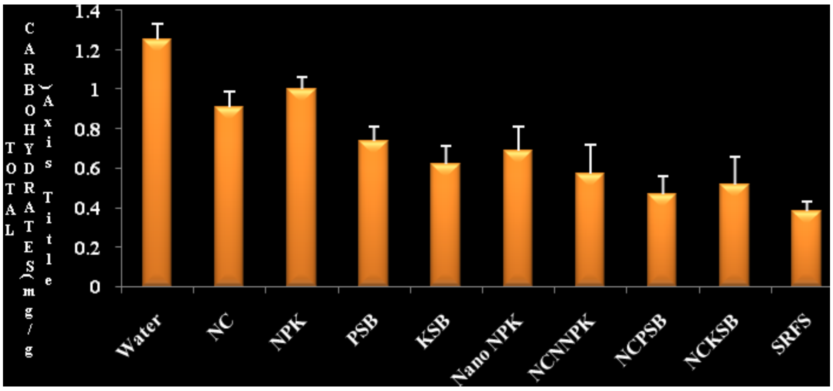

Figure 8. Influence of SRFS on Total carbohydrates of *Vigna radiate*

Conclusion

The present study confirms that fertilizers in nano formulation can be fabricated into slow release fertilizers system with biocompatible non toxic organic carrier neem cake. Use of PGPR facilitates the efficient use of NPK fertilizers. Germination and seedling growth is enhanced by accelerating the battery of enzymes.

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