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RESEARCH ARTICLE

EVALUATION OF HUMUS AS CARRIER FOR BIOFERTILIZERS TO IMPROVE THE GROWTH OF VEGETABLE CROPS AND SOIL FERTILITY.

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Abstract

The aim of the present study was to evaluate the potential of humus as carrier for PGPR isolates to improve the growth of crop plants. The NFB, PSB and KSB isolates separately and in combination were mixed with humus and cured. The shelf life of PGPR in the carrier based preparations were monitored upto 6 months and found to be maximum at 105th day for PSB & KSB and 120th day for NFB. At 180th day no growth was obtained. But the carrier incorporated with 10% sucrose showed microbial growth after 210 days. The effect of biofertilizer preparations were tested on brinjal seedlings at 7 treatments in 5 replications. Co-inoculated pots were reported with better growth rate when compared to control pots. The soil analysis showed significant increase in plant available nutrients in soils treated with coinoculation of NFB, PSB and KSB. From this study, it is concluded that humus can be used as an effective, ecofriendly and long lasting carrier for bioinoculum to be used as bioferlilizer to improve the growth of vegetable crops as well as soil fertility.

Introduction:-

Soil is a great reservoir of plant nutrients. But most of these reserves may not be in plant available form. So it is necessary to analyse plant available soil nutrients for effective nutrient management and crop production systems (Motsara, M.R. and Roy, R.N.,2008). Application of fertilizer is important in modern agriculture to maintain soil health thereby achieving higher crop yield. Chemical fertilizers can enhance the growth and productivity of food crops within short period of time, but they are expensive and leads to serious environmental issues. Usage of biofertilizer instead of chemical fertilizer is the most desired practice for sustainable agriculture. Biofertilizers are living microorganisms that help crop plants to access the nutrients available in the rhizosphere. In india commercialization of biofertilizer started in the late seventies and presently they are produced in an industrial level (Brar et al.,2012). Carrier based biofertilizer technology uses a large number of carriers including charcoal, peat, press mud, lignite, vermiculite etc. But still works are going on to explore cheap and efficient carrier for biofertilizer with better shelf life.The carrier based biofertilizer technology involves two steps; the production of microbial biomass in a phased manner to desired level and the pure culture broth is mixed with carrier material and is packed in polythene bags under sterile conditions (Yadav A K and Chandra K., 2014). Humus has a potential to be used as biofertilizer carrier because of its positive influence on soil fertility and plant growth. Humus acts like a big sponge that can hold upto 90% of its weight in water. This water holding capacity makes humus moist for weeks. Also because of its negative charge it holds many of the plant nutrients such as ammonium, calcium, magnesium,

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phosphorus etc and prevents rain from washing them away. When the plant roots are in contact with it, the nutrient will be easily accessed by the plant roots (Robert Palvis.,2013). This work describes the use of humus as a carrier for nitrogen fixing (NFB), phosphate solubilizing (PSB) and potassium solubilizing bacteria (KSB) to improve the growth of brinjal plants in pot experiments.

Methods:-

Preparation of carrier and production of biofertilizer

Humus samples were collected manually using gloves at a depth around <5cm from the woods near Kothamangalam, Ernakulam district of Kerala. It was dried at 45°C, powdered and sieved. The physicochemical properties of humus was tested in a soil testing laboratory.The pH of the humus was adjusted to 6.5 by adding wood ash to favour the growth of bacteria. About 6 low density grade polythene bags were filled with 200 g carrier substrate and sterilized at 121°C for 3 hours. The sterilized carrier bags were inoculated with 40 ml suspension of 3-4 days old inoculum of NFB, PSB, KSB separately and in combination. The bags were incubated for 2-3 days in open at room temperature in a sterile room. Then the bags were sealed and stored in aseptic condition for a period of 6 months. The carrier without inoculum was served as control.One of the carrier bags was incorporated with 10% sucrose and co-inoculated with 3 strains.

Shelf life of biofertilizer:-

At every 15 days biofertilizer samples were withdrawed from bags for microbial analysis. NFB, PSB and KSB were enumerated by dilution plate method on specific media to check the shelf life of biofertilizer. DF minimal agar (Dworkin and Foster, 1958), Pikovskayas agar (Pikovskaya, 1948) and Aleksandrovs agar (Hu et al., 2006) medium was used for NFB, PSB and KSB respectively. The inoculated strains showed an average viable count of 12.3×10^8/ml for NFB, 5.2×10^8/ml for PSB and 4.5×10^8/ml for KSB.

Effect of biofertilizer preparations on growth of Brinjal seedlings in pot experiment:-

The biofertilizer preparations were applied to pots with sterile soil planted with brinjal seedlings in a ratio of 50g/2kg soil. Seven treatments were made in 5 replications (control, carrier control, carrier based NFB, PSB, KSB and NFB+PSB+KSB). All the treatments were sprinkled with water daily to ensure good growth of bacteria. The growth of brinjal plants were monitored at 15 and 30 days and compared with control. Plant growth parameters tested are shoot length, root length and number of leaves.

Soil Analysis:-

After harvesting plants the soil samples (250 gm each) were collected, dried, crushed and passed through 2mm sieve. These samples were analysed for available nitrogen content by Alkaline Permanganate Method (Subbiah and Asija.,1956), available phosphorous content by colorimetry (Olsen et al.,1954) and available potassium content by flame photometry (Black C.A., 1965).

Data Analysis:-

All the results were analysed using the software SPSS Version 16. A two way mixed ANOVA was performed to examine the effect of treatment and duration on shoot length, root length and number of leaves. A one way between subjects ANOVA was conducted to compare the effect of treatments on N, P and K level in soil samples. Post hoc analysis using Bonferroni correction was used to find out the significant difference between the treatments.

Results and Discussions:-

Preparation of carrier and production of biofertilizer:-

The physicochemical properties of the humus sample is presented in table 1.

Table 1:-Properties of humus sample

Sample	pH	C	N	K	Ca	Fe	Mg	Na	Al
Humus	5.5	25	1.5	14	250	0.2	50	2.8	1.1

*C and N expressed in % dry weight; K, Al, Ca, Fe, Mg and Na expressed in mmol/kg

Biofertilizer formulation containing NFB, PSB and KSB were prepared using humus as carrier material and was compared with FCO, 1983 and tabulated in table 2.

Table 2:-specification of biofertilizer preparation.

Sl. No.	Parameters	New biofertilizer preparation	Reference (FCO, 1983)
1	Base	Carrier based (moist/drypowder)	Carrier based (moist/dry powder or granules) or liquid based
2	Viable cell count	CFU >5×10^7cell/g of Carrier material	CFU minimum 5×10^7cell/g of powder, granule or carrier material or 1×10^8cell/ml of liquid
3	Contamination level	No contamination at 10^5diluion	No contamination at 10^5diluion
4	pH	6.8	6.5-7.5
5	Particle size	0.2mm	All material shall pass through 0.15- 0.212 mm IS sieve
6	Moisture percent by weight	40%	30-40%
7	Efficiency character 1. Free living N fixers 2. Phosphate and Potassium solubilizing bacteria	Fixed 15mg of N /g of sucrose consumed 12mm zone for PSB(11mm thickness) 10mm zone for KSB(9mm thickness)	1.The strain should be capable of fixing at least 10mg of N/g of sucrose consumed 2&3. Minimum 5mm solubilization zone in prescribed media having at least 3mm thickness

The shelf life of Biofertilizer:-

The viability of PSB and KSB in sterilized humus showed a maximum positive result upto 105th day and NFB upto 120th day. After that the viable count of strains showed a downward effect. At the end of 180th day no viable strains were observed in all the carrier bags except the one with 10% sucrose. This may be due to the depletion of nutrients, moisture and autolysis of cells during prolonged storage (Gaind and Gaur 1990). The carrier bag incorporated with 10% sucrose showed viable cells after 210 days. Enrichment of the carrier material with nutrients such as sucrose, maltose, glucose, glycerol, molasses etc ensure maximum cell viability and extended shelf life by allowing the microorganism to maintain or grow in a noncompetitive micro environment (Yardin et al., 2000).

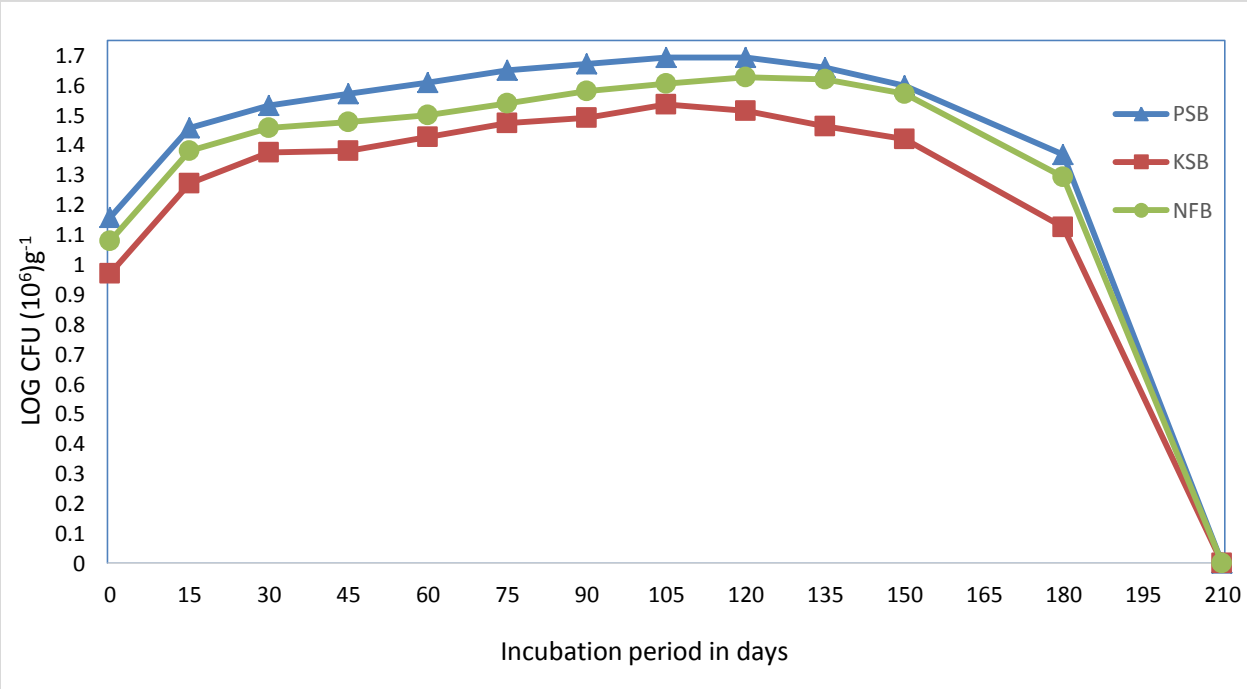

Fig. 1:– Shelf life of PGPR in carrier substrate (Individual treatments)

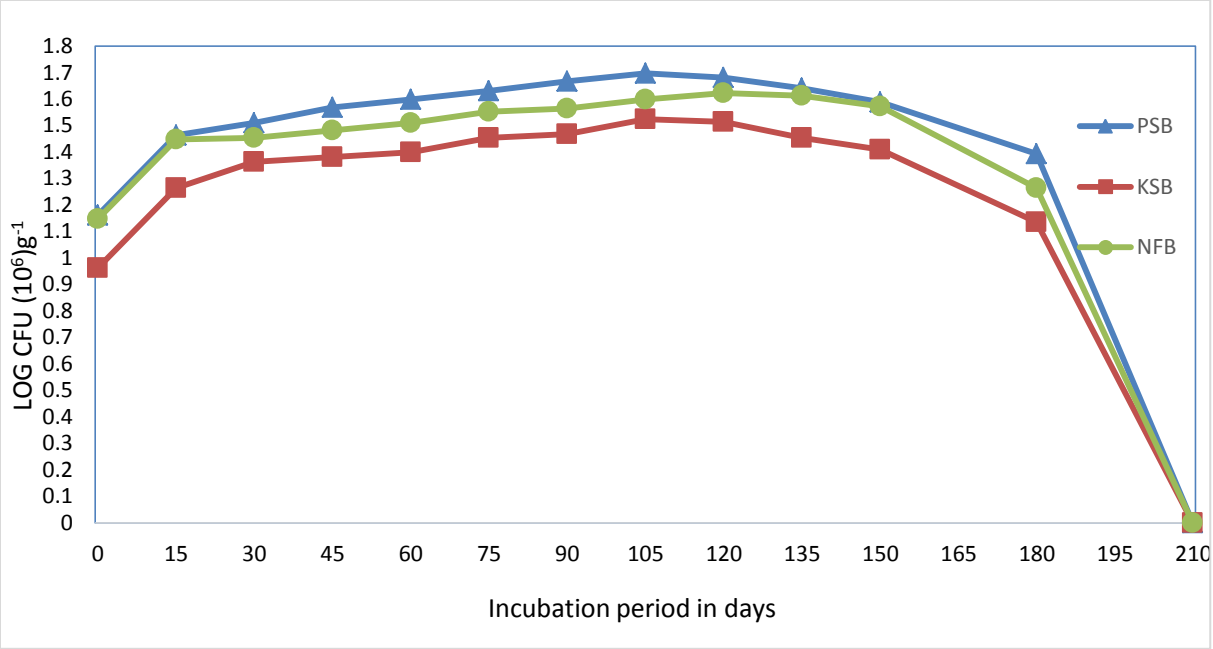

Fig. 2 :- Shelf life of PGPR in carrier substrate (Combined treatment without sucrose)

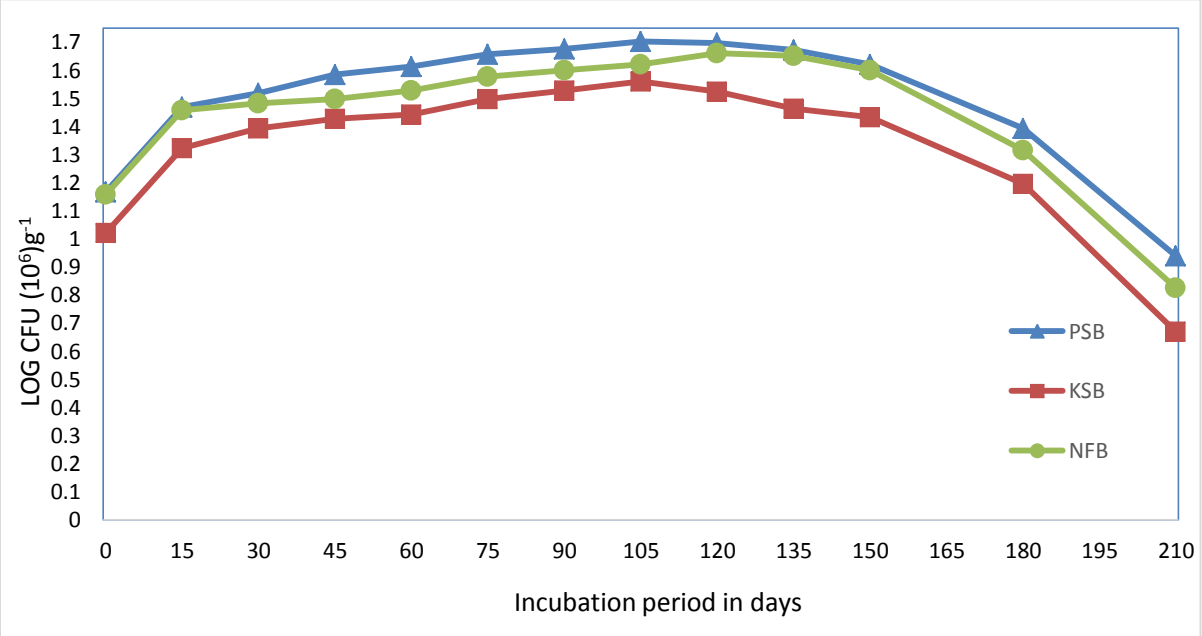

Fig. 3:-Shelf life of PGPR in carrier substrate (Combined treatment with sucrose)

Effect of biofertilizer preparations on growth of Brinjal seedlings in pot experiment:-

Co-inoculation with NFB, KSB and PSB was found to be the most effective treatment to improve the growth of brinjal seedlings when compared to the control treatment. Separate inoculation of NFB, KSB and PSB also had a positive effect on brinjal seedlings but less than that of co inoculation treatment. Based on Greenhouse – Geisser correction, there was a significant effect of duration on shoot and root length and number of leaves ($P<0.001$). Dual inoculation with AMF and rhizobacteria was improved the growth and nutrient uptake in maize plants were reported by Wu et al., 2005. PGPR not only provides nitrogen, phosphorous and potassium available to plants but also produces a variety of plant growth promoting substances such as indole-3-acetic acid, cytokinines and gibberellins (Van Loon., 2007; Contreras-Cornejo et al., 2009).Hall et al.,1996; Glick et al.,1997 observed the shoot and root elongation of Canola, Lettuce and Tomato on inoculation with *Pseudomonas*.

Table 3 :- Growth rate of brinjal plants after 30 days

Treatments	Shoot Length(cm)	Root Length(cm)	No of Leaves
Control	14.50 ± 0.41	3.28 ± 0.16	4.60 ± 0.55
Carrier control	19.50 ± 0.38	3.70 ± 0.40	5.40 ± 0.55
Carrier-NFB	24.12 ± 0.74	7.58 ± 0.38	6.20 ± 0.84
Carrier- PSB	22.98 ± 1.09	6.66 ± 0.40	6.00 ± 1.00
Carrier- KSB	23.32 ± 0.44	5.78 ± 0.19	5.20 ± 0.84
Carrier- NFB+PSB+KSB	**29.30 ± 0.45**	**9.26 ± 0.21**	**7.60 ± 0.55**
Chemical Fertilizer	24.36 ± 0.42	7.82 ± 0.84	6.60 ± 0.55

Soil Analysis:-

The combined inoculation of 3 rhizobacteria resulted in a significant increase in soil available nutrients than individual treatments and chemical fertilizer. The available N, P and K content (Kg $ha^{-1)}$ in the soil with combined inoculation was found to be; 578 ± 1.67, 40 ± 1.00 and 648 ± 1.30 respectively.

Table 4 :– N,P & K Content of soil samples

Treatments	N (kg ha^{-1})	P (kg ha^{-1})	K (kg ha^{-1})
Control	249.00± 1.00	04.20 ± 0.84	179.20 ± 0.84
Carrier control	254.20 ± 0.84	05.20 ± 0.84	189.20 ± 0.84
NFB	498.80 ± 1.30	17.80 ± 1.30	208.80 ± 1.30
PSB	298.80 ±1.30	33.80 ± 1.30	198.80 ± 1.30
KSB	288.80 ± 1.30	08.80 ± 1.30	548.80 ± 1.30
NFB+PSB+KSB	**578.60 ± 1.67**	**40.00 ± 1.00**	**648.80 ± 1.30**
Chemical fertilizer	563.80 ± 1.30	27.40 ± 1.14	598.80 ± 1.30

This result indicated that there is a significant increase ($p<0.001$) in soil nutrient level by the application of biofertilizer. Biostatistically there was a significant effect of treatments on N,P and K concentration levels in soils for all the groups ($P<0.001$). Post-hoc comparisons using Bonferroni correction showed that the mean N, P and K concentration level was significantly different for all the treatments ($P<0.001$). Microbial fertilizers convert plant nutrients from an unavailable to available form by a number of biological processes (Vessey., 2003).

Conclusion:-

Nowadays Biofertilizer technology seeks more attention as it help developing organic farming and sustainable agriculture. When using carrier based biofertilizer, the carrier material should not have any harmful effect on soil and environment. Humus is such an ecofriendly material rich in plant nutrients. The humus based biofertilizer showed better shelf life and its treatment with brinjal plants exhibited significant growth response under pot trials. Also an increased level of plant available form of nitrogen, phosphorus and potassium were reported in the soil samples. Soil bacteria is an excellent system for substituting chemical fertilizers. This study strongly supports the use of humus as carrier for co-inoculating NFB, PSB and KSB, which not only promote plant growth but also build up a healthy nutrient rich soil.

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