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Research Article

EVALUATION OF FORMULATIONS AND THEIR IMPACT ON PHYSIOLOGICAL CHARACTERS OF GREEN GRAM (*VIGNA RADIATA*): A POT EXPERIMENT

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ABSTRACT

This study was done to determine the effect of formulations with traditional fertilizers (cow dung and vermi-compost) on root and vegetative growth of green gram under *in-vitro* condition. The various treatments were done by formulation I, formulation II, Co-formulation (for pending patent, Patent App. No 201741023234), whey, molasses and formulations with traditional fertilizers in various ratios and applied as three replicates in randomized complete design. Results showed that all physiological characters of treated plants were significantly increased. But the pots treated with co-formulation and co-formulation with vermi compost in 1:3 ratio gave the highest results in shoot and root length, number of leaves and branches on 30th day of plant germination. This improved growth occurred is mainly due to the nutrient availability in bio-organic fertilizers and up taken by plants.

Keywords: Bio-organic fertilizers, Molasses, *Pseudomonas fluorescens*, Traditional fertilizers, *Trichoderma viride*, Whey

1. INTRODUCTION

The green revolution has led to intensified agriculture to meet the ever increasing demands for food and fiber which is practiced at great cost to the environment, resulting in continuous damage of natural ecosystems, ground water and food-stuff pollution and other environmental degradation. Haphazard use of chemical pesticides and fertilizers in modern agriculture has resulted in the development of several problems such as pesticide resistance in pests, resurgence of target and non-target pests, chemical residues in food, feed and fodder and destruction of beneficial organisms like honey bees. Awareness about the health hazards and environmental problems due to the continuous use of pesticides resulted in the development of Integrated Pest Management (IPM). Keeping this in view, studies have been initiated to include crop protection and growth promotion using the native micro-organisms as a component of PGPR [1].

Bio-fertilizers are the biological decomposition of organic materials; also known as the cultures of microorganisms like bacteria, fungi and blue green algae, which directly give nutrition to crop plants; packed with a carrier material. These organisms are added to the rhizosphere of the plant to enhance their activity in the soil to get high yield. Hence, it is necessary to look for alternative disease management practices, which include the use of eco-friendly biological control agents (BCAs) and pathogen-resistant crop cultivars. The "microbial inoculants" can generally be defined as preparation containing live or latent cells of efficient strains of nitrogen fixing, phosphate solublizing or cellulolytic microorganisms used for application to seeds, soil or composting areas with the objective of increasing the number of such microorganisms and accelerate those microbial process which augment the availability of nutrients that can be easily assimilated by plants. Biofertilizer can provide an economically viable support to small and marginal farmers for realizing the ultimate goal of increasing productivity. Biofertilizers are low cost, effective and renewable source of plant nutrients instead of chemical supplements.

Pseudomonads (Fluorescent) are considered to be the most promising group of plant growth promoting rhizobacteria involved in biocontrol of plant diseases [2]. They produce secondary metabolites such as antibiotics, phytohormones, volatile compound Hydrogen cyanide (HCN) and siderophores. Plant growth-promoting ability of these bacteria is mainly because of the production of indole-3-acetic acid [3], siderophores and antibiotics [4].

Trichoderma spp. has evolved numerous mechanisms that are involved in attacking other fungi. These mechanisms include competition for space and nutrients, mycoparasitism, production of inhibitory compounds [5, 6] inactivation of the pathogen's enzymes [7] and induced resistance. Today, more than 50 different *Trichoderma* based agriculture products can be found as registered in many countries; are sold and applied to protect plants and soil and for improving yield of vegetables, ornamentals and fruit trees. *Trichoderma* is completely safe and in 55 years of research there has never been a recorded adverse reaction on humans and livestock [8].

Among different BCAs, *Pseudomonas* spp and *Trichoderma* spp have proved effective and selective enough against most of the various fungal and bacterial diseases. The various currently used biological approaches, use of microbial inoculants at the seedling stage could prove as a promising approach. Several symbionts like arbuscularmycorrhizal (AM) fungi, *Trichoderma* spp, a known biocontrol agent, and phosphorus solubilizing bacteria (PSB) like *Pseudomonas fluorescens* can be implemented for green gram cultivation.

The best way to reduce substrate cost for microbiology at present is to use wastes with the right balance of carbohydrates and lipids to support optimum bacterial growth and which are very effective as well as free of cost. As is known, millions of tons of hazardous and non-hazardous wastes are generated each year throughout the world. The several renewable substrates include various agricultural and industrial by-products and waste materials have been intensively studied for microorganism cultivation at a laboratory scale, for example: olive oil mill effluent, waste frying oil, oil refinery wastes, soap stock, molasses, whey, starch wastes, cassava flour processing effluent and distillery waste [9].

Green gram is one of the most important pulse leguminous crops. It is grown in almost all parts of the country. This was reported earlier in India for the cultivation of green gram plant. It is an excellent source of high quality protein rich staple food. It contains about 25 percent protein which is almost three times that of cereals and consumed in different ways as dal, halwa, snack and so many other preparations. It supplies protein necessity of vegetarian inhabitants of the country. Ascorbic acid (vitamin C) is synthesized in sprouted seeds of green gram with increment in riboflavin and thiamine [10].

The establishment of *Pseudomonas fluorescens* and *Trichoderma viride* in rhizosphere and their effect on the growth of green gram was determined in garden soil by pot cultivation. The ultimate aim of this study is to examine the influence of formulation I, formulation II, co-formulation of formulation I and II, various combinations of formulations with traditional bio fertilizers such as cow dung and vermi-compost on green gram. In this pot cultivation study, the various physical characteristics of growth such as shoot length, root length and number of leaves and branches were measured and counted.

2. MATERIALS AND METHODS

2.1. Pot culture experiment

The formulation I and formulation II were prepared using a particular combination of liquid whey and molasses in aseptic conditions. The earthen pot culture experiment was carried out in the campus of MYRADA KVK located at Gobichettipalayam, Erode.

1.1.1. Soil

90-95 kg of soil was sterilized at 15 lbs pressure at 82°C for 4 hours.

1.1.2. Pots

Pots were selected for the experiment and these pots were sterilized by using formalin (surface sterilization), and it is filled with sterilized garden soil.

1.1.3. Inocula (Various combinations of *Pseudomonas fluorescens* and *Trichoderma viride*) [1]

The formulations and different combinations of formulations with traditional fertilizers were prepared in aseptic condition.

The inoculums are:

- Formulation I (FI)
- Formulation II (FII)
- Co-formulation of formulation I & II (CF)
- Nine combinations of formulations with cow dung
- Nine combinations of formulations with vermi-compost
- Whey liquid (WL)
- Molasses (M)
- Cow dung liquid (CL)
- Vermi-liquid (VL)

1.2. Seed treatment

The small oval healthy viable green gram seeds were selected for pot culture experiment. One hundred mL of all inoculums were prepared in aseptic conditions. 3mL of each inoculums was mixed with 3ml of cool rice porridge separately. The seeds were then mixed with its respective inoculated rice porridge to form a bacterial and fungal coat on the surface of green gram. Finally, the seeds were allowed to dry under shade for 30 minutes and sown the seeds within 24 hours.

1.3. Growth characters of plants

Ten seeds were sown in each pot. The control pots were devoid of various formulations. All the pots were flushed twice with water daily. Ten days once (day 1st - seed treatment, 11th and 21st) the treated pots were fertilized with 20 mL (20 mL was mixed with 80 mL of water to spread the inoculums evenly in pots) of its respective inoculums (2mL/plant). The seeds were allowed to grow till 5 days. Five days once (5th, 10th, 15th, 20th, 25th and 30th), the physical parameters of green gram plants were checked up to 30th day.

The plants were uprooted gently without disturbing the root system and the roots were washed with tap water to remove soil particles. Then the height of seedlings (shoot lengths) from surface of the soil to the shoot tip and the lengths of roots from surface of the soil to the root tip were measured in meter scale. The number of leaves and branches were also counted and tabulated.

Ten healthy green gram seeds were sown in each pot. The pots were watered well. The control pot was devoid of various formulations. The experimental pots were fertilized with formulations (I, II and III) combined with various traditionally available biofertilizers like Vermi compost (VC) and Cow dung (CD) (Fig. 1). The effect of formulations on the growth of plant roots and shoot length are measured, root nodules, leaves and branches were observed and counted on every five days once upto thirty days. Length and width of leaves of ten randomly selected plants from each treatment were measured with a meter scale from the end of petiole to the apex of the leaf. Root lengths of selected plants from each treatment were measured with a meter scale.

The treatments were distributed in a randomized design with three replications. In each crop three treatments (Formulation I, II and III) were analyzed. The liquid bio-fertilizer was designed by our own idea from whey and molasses combinations i.e. formulations and we just compared commercial biofertilizers i.e. cow dung and vermi compost for the growth of green gram plant. The detailed combinations were shown in Fig. 2 and 3.

2. RESULTS AND DISCUSSION

Multiple strains mixtures of microbial agents have been used successfully for the plant growth in earlier studies. Based on this research, organic fertilizer based formulations containing a mixture of bacteria and fungi were tested and recoded the physiological growth of green gram plant. The detailed results were discussed in below.

2.1. Pot culture experiment

In this experiment, various treatment trails (formulations, formulations with traditional fertilizers, liquid whey and molasses) were tried on green gram germination. After sowing, five days once various characteristics of growth such as shoot and root length, number of leaves and branches were measured up to thirty days and the results were tabulated. All treated pots showed positive effects. The untreated pots represented as controls.

Plate 1: Characterization of physical parameters from Pot culture method

A) Adding Inoculums (Formulations); B) Root Nodules; C) Shoot Length; D) Root Length

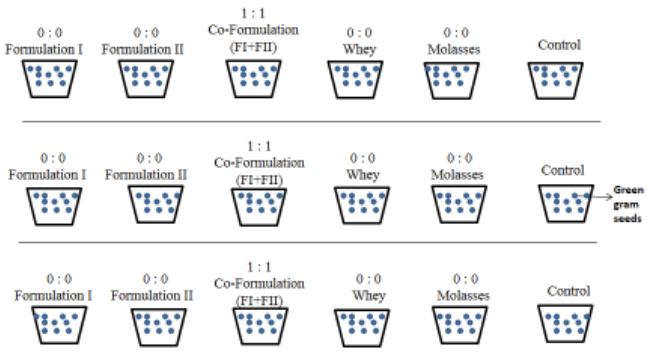

Fig. 1: Effect of Formulations on green gram germination in pot cultivation Comparative study on growth and germination of green gram using biofertilizers

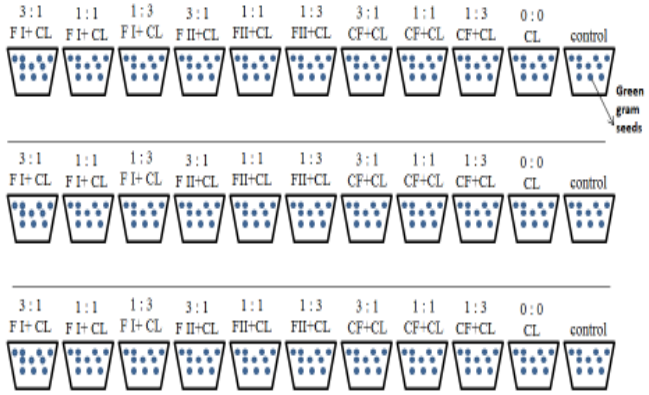

Fig. 2: Effect of various combinations of formulations with cow dung on green gram germination in pot cultivation

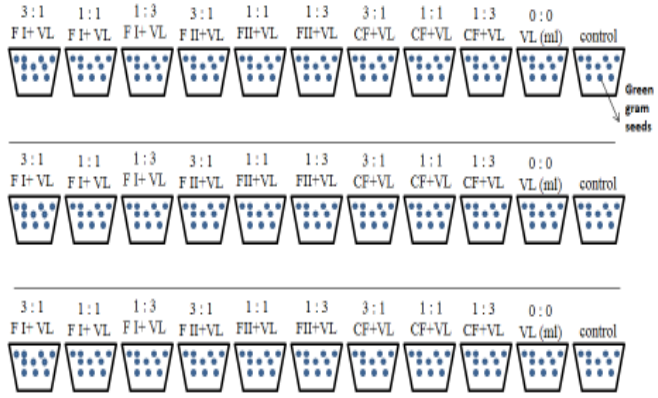

Fig. 3: Effect of various combinations of formulations with vermi-compost on green gram germination in pot cultivation

The fig. 4 shows that the highest shoot length was observed in the pots treated with 1:3 ratio of co-formulation combined with vermicompost. When compared to the highest shoot length, only there were slight difference occurred in pots treated with co-formulation and co-formulation combined with cow dung in 1:3 ratio. The pots treated with formulation I & II and formulations combined with cow dung and vermicompost as individual were taller than the remaining pots revealed. In meanwhile, whey treated plant had attained better growth rate than molasses and control. Minimum shoot lengths were recorded in all control pots.

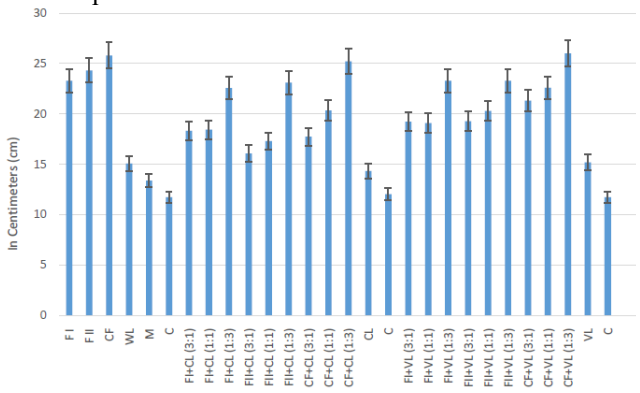

Fig. 4: The effects of formulations and formulations with traditional fertilizers on shoot length (30th day)

From F I + CL, F II + CL & F III + CL combinations (3:1; 1:1; 1:3), all the formulations were observed better shoot growth of green gram plant at 30th day. Significant increases in shoot lengths of the plants were recorded particularly in the triple inoculations compared with all the practices (Fig. 1 & Plate 1).

The worms along with organic manure's can be utilized as an alternative to costly inorganic fertilizers. Microorganisms have developed the mechanisms to cope with a variety of toxic metals for their survival in the environment enriched with such metals. This study observed few rhizobacteria tolerant to multiple heavy metals and exhibiting a couple of PGP activities Pseudomonas sps were resistant to 400 gml^{-1}. It was also apparent that more cultures of PGPR isolated from rhizosphere were tolerant to elevated levels heavy metals. The metal tolerant microorganisms are selected for the growth as well as the recolorization of the plant rhizosphere in polluted soils [12].

In the mid-1980s, measurement of root volume became popular as a means of evaluating whole root system. There are many positive nutritional relationships between root and field survival microorganisms. High degrees of variability in root competition within relatively small areas suggest local processes, variation in root volume maybe at least as important in determining individual plant growth, how the mean

strength of competition can vary with productivity [13].

The highest root length was achieved through application of 1:3 combination of co-formulation combined with vermicompost. Similarly significant higher root length was observed in pots treated with co-formulation and co-formulation combined with cow dung in 1:3 ratio. The pots treated with remaining treatments showed remarkable effect on root length than control pots of green gram.

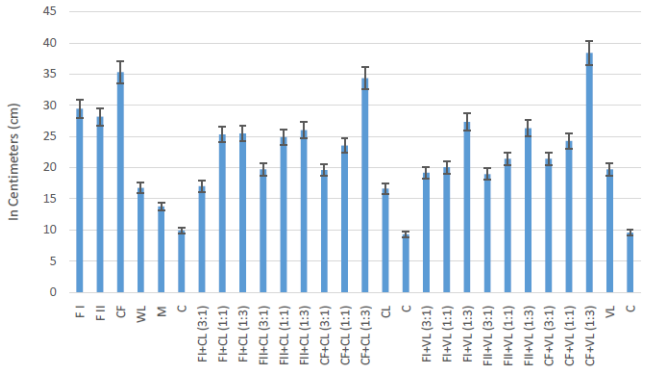

Fig. 5: The effects of formulations and formulations with traditional fertilizers on root length (30th day)

All the combinations were significantly increasing their efficiency on the growth roots. Here, this combination F I, II & III (1:3) showed the best performance at the end of 30th day seed germination than others and control. From these results, formulations (I, II & III) were observed better results than others and control. In mean while whey and molasses mean values of triplicates slightly differ when compared all the formulations (I, II & III).

In this study, root volume increased significantly in every plant which is treated with various formulations (FI, FII & CF) than control. Plants that treated with CF+CL (1:3) as well as CF+VL (1:3) have the highest root volume because of water extractable regulators on initial root development and plant germination. FI+CL (1:3) and FII+CL (1:3) have recorded as moderate height of root system than CF+CL (1:3). Similarly, CF+VL (1:3) had noticed highest root volume than FI+VL (1:3) and FII+VL (1:3) (Fig. 5 & Plate 1). The other treatments were comparable with control. The maximum root length was provided by vermi compost liquid (VL) with co-formulation (CF) and closely followed by cow dung liquid (CL) with co-formulation (CF). The findings were in confirmation with the observations of earlier report [12] as they also noticed that traditional fertilizer with PGPR organisms enhanced plant growth and nutrient uptake as compared to control.

The maximum number of leaves was achieved by the pots treated with formulation I, formulation II, co-formulation, formulations and co-formulation combined with cow dung and vermicompost in 1:3 ratio as individual (Fig. 6). There were also pronounced effect in the pots fertilized by other combinations of formulations with traditional fertilizers, whey liquid and molasses. The lowest number of leaves occurred in control plants.

Same results were observed for the combination of vermi compost and formulations. The increase in leaves number due to the application of organic components stimulatory effects on cell division and enlargement, protein and nucleic acid synthesis. Non poured plants had the lowest population, followed by plants poured with various combinations of traditional biofertilizer & formulations and were comparable. According to Khalizadeh *et al.*, (2012) [14] the foliar application of urea and bio-organic fertilizers have a positive impact on total plant germination.

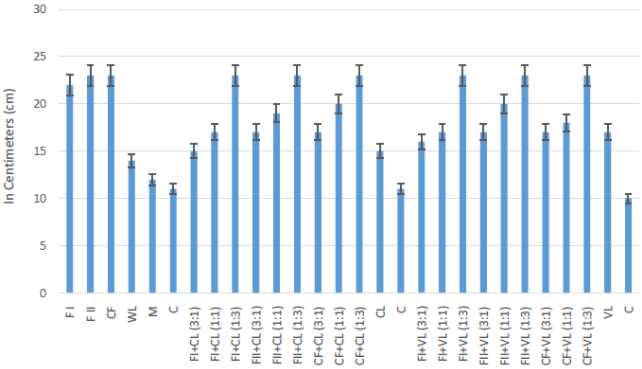

Fig. 6: The effects of formulations and formulations with traditional fertilizers on number of leaves (30th day)

The maximum number of branches was counted in plants influenced with 1:3 combinations of FI combined with vermicompost, FII combined with vermicompost and co-formulation combined with vermicompost. The significant effect on branches of green gram was observed on the pots treated with formulations and combinations of formulations, whey and molasses. Lowest numbers of branches were counted in control pots.

The number of branches in green gram plants increased significantly in treatments with CF+VL (1:3) and it was followed by CF+CL (1:3) (Fig. 7). Furthermore, Zaky *et al.*, (2006) [15] found that the number of shoots/plant were increased in bean plants by application of traditional fertilizer with formulations at a ratio of 3:1 &1:1

appeared to have lowest stimulatory effects on shoot growth as compared to control and other treatments.

From these results, all the combinations were significantly increased the number of branches in green gram plant during seed germination. Here, these combinations F I, II & III (1:3) showed the better at the end of 30th day seed germination than others and control. From the above results, all the combinations were significantly increased the number of branches in green gram plant during seed germination.

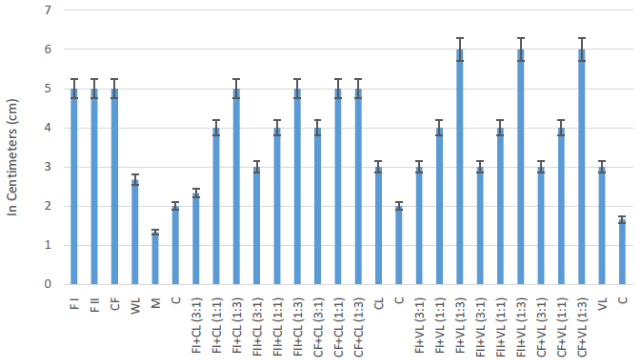

Fig. 7: The effects of formulations and formulations with traditional fertilizers on number of branches (30th day)

3. CONCLUSION

The results of present study clearly indicate that the selected organisms (*Pseudomonas fluorescens* and *Trichoderma viride*) which were applied on green gram with composite ratio of 1:3 were highly beneficial for enhancing the yield besides effecting a reduction in the cost of inorganic fertilizers. When seeds treated with various formulations showed significant increase in growth parameters of green gram plant. Their morphological parameters such as shoot and root length and number leaves and branches of the plants observed the excellent growth and significantly increased in all the experimental plants. All the physical parameters have been increased in experimental plants than controls.

4. ACKNOWLEDGEMENT

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Conflict of interest

On behalf of all authors, the corresponding author states that there is no conflict of interest.

5. REFERENCES

1. Ganesan S., Sekar, R. (2004a). Biocontrol mechanism of Groundnut (*Arachishypogaea* L.) Diseases-Trichoderma system. In: Biotechnological Applications in Environment and Agriculture (Eds. G.R. Pathade and P.K. Goel), ABD Pub. Jaipur, India. 312-327.
2. Moeinzadeh A, Sharif-Zadeh F, Ahmadzadeh M, and Heidari Tajabadi F. *Aust. J. Crop Sci.*, 2010; **4:**564-570.
3. Patten C, Glick B. *Appl. Environ. Microbiol.*, 2002; **68:**3795-3801.
4. Keel C, Schnider U, Maurhofer M, Voisard C, Laville J, Burger U, Wirthner P, Haas D, Defago G. *Mol. Plant-Microbe Interact*. 1992; **5:**4-13.
5. Sivasithamparam K, Ghisalberti EL. (1998). Secondary Metabolism in Trichoderma and GliocladiumIn: Trichoderma and Gliocladium Basic Biology, Taxonomy and Genetics, Kubicek C.P., G.E. Harman and K.L. Ondik (Eds.). Taylor and Francis. 139-191. ISBN: 0748405720.
6. Elad Y, David DR, Levi T, Kapat A, Kirshner B. 1999. *Trichoderma harzianum* T-39-mechanisms of biocontrol of foliar pathogens. In: Modern fungicides and antifungal compounds II. Eds. H. Lyr, P.E. Russell, H.W. Dehne, and H.D. Sisler). Andover, Hants, UK: Intercept. 459-467.
7. Roco A, Perez LM. *Electronic J. Biotechnol.,* 2001; 4. Available from http://www.ejbiotechnology.info/ntent/vol4/issue2/full/1/1.pdf.
8. Anonymous. 2005. Commercial biocontrol products available for use against plant pathogens.
9. Makkar RS, Cameotra SS. *Appl. Microbiol. Biotechnol.*, 2002; **58:**428-434.
10. Pandey PH. 1988. Principles and Practices of Post Harvest Technology.
11. Nanthini PVM, Shahitha S, Palaniswamy M. *International Journal of Basic and Applied Research*, 2019; **9**.
12. Shanmugaiah V, Manoharan PT, Rajendran A, Mathivanan N. *Ind. J. Bot. Res.*, 2008; **4(1):**99-110.
13. Shanmugaiah V, Balasubramanian N, Gomathinayagam S, Manoharan PT, Rajendran A. *African Journal of Agricultural Research* , 2009; **4(11):**1220-1225.
14. Khalilzadeh R, Tajbakhsh M, and Jalilian J. *International Journal of Agriculture and Crop Sciences*, 2012; **4(10):** 637-642.
15. Zaky LM, Hassanein RA, Dowidar AE, El-Mashad AA. *Egypt. J. Physiol. Sci.*, 1999; **23(3):**443-469.