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EFFECT OF BIO-STIMULANTS ON GROWTH AND YIELD OF COWPEA (*Vigna unguiculata* (L.) WALP)

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Cowpea (*Vigna unguiculata* L. Walp), is an important semi-arid legume crop mostly cultivated in the warmer regions of Indian Sub-Continent. It is a rich source for dietary fibers, proteins, vitamins and essential minerals (Goncalves *et al*., 2016; Raguraman *et al*., 2019). The crop is utilized either for dry seeds (or) green pods (Abbas and Akladious, 2013). The seeds of cowpea have known to reduce the cholesterol as it contains high dietary fiber and high protein content (Frota *et al*., 2008). Apart from this the rich antioxidant properties of cowpea diminish the cancer cells initiation and growth (Patel *et al*., 2018). It is popularly called as vegetable meat as it plays an important role in Indian diet on account of high per cent of proteins (23.14%), which is double than that of cereals. It can grow and thrive well in low fertile soils due to symbiotic nitrogen fixation (*Rhizobium* sp.) thus helps to maintain sustainable agricultural farming (Zahran, 1999).

Extensive use of chemical fertilizers results in reduced growth of soil microbial community and thereby reduced soil fertility (Chen, 2006). The use of organic biofertilizer should be the proper alternative to overcome the detrimental effects of chemical fertilizers.

Use of bio-stimulants instead of chemical fertilizers improves the soil and water quality (Maghirang, 2011). These favorable conditions potentially enhance the plant growth and yield (Judith *et al*., 2017). Bio-Stimulants are mainly composed of different vitamins, antioxidants, hormones, enzymes, sugars and amino acids (Thirumaran *et al*., 2009). Jeevamrutha, sea weeds and KNF are some of the biostimulants generally used as biofertilizers.

Jeevamrutha improves the soil health with an average nutrient content of nitrogen 1.48%, phosphorus 0.28% and potassium 0.32% (Vala and Chavda, 2021). Jeevamrutha spray at a rate of 200-500 liters/acre in field bean improved the yields up to 40% (Anon, 2010). Reshma *et al*. (2015) claimed the increased growth and yield of cowpea on Jeevamrutha application. Seaweeds are important marine sources of the world with an average nutrient content of nitrogen 1.2%, phosphorus 4.0% and potassium 2.1% (https://nutrientoptimiser.com) and are available in the form of liquid fertilizers and powder (Booth, 1969). The Foliar application of sea weed S*argasssum crassifolium* extract on tomato plants was earlier reported by Sutharsan *et al*. (2014). Enhance yield of brinjal on treatment with *Stoechospermum marginatum* extracts was previously reported (Sivasangari *et al*., 2015). Korean Natural Farming (KNF) is a sustainable system that maintains nutrient balance. The average nutrient content of KNF is recorded as 4.05% (nitrogen), 7.48% (phosphorus) and 5.22% (potassium) (Keliikuli *et al*., 2019). KNF encourage the growth of naturally occurring indigenous microorganisms (IMO). The influence of different KNF treatments on plant growth and yield traits were earlier documented (Dabbaghi *et al*., 2018).

Even though biostimulants drives the crop growth, very few studies are available (Yakhin *et al*., 2017; Rouphael *et al*., 2018a). Hence the present study was carried out to establish a clear envisage of bio-stimulants effects on growth and yield potentiality of cowpea.

Experimental design

A pot culture experiment was conducted during *Kharif*, 2021 at Botanical Garden of SRR & CVR Government Degree College, Vijayawada, Andhra Pradesh. A dwarf variety of cowpea (Khasi Kanchan) was obtained from Indian Institute of Vegetable Research (IIVR), Varanasi.

The experiment was conducted in complete randomized block design with five replications. Three seeds for each genotype were sown on first week of November in forty earthen pots (30 × 30 cm) having red loamy soil. Watering was done regularly and finally having one plant in each pot. All the pots were categorized in to 4 sets with 10 pots per set. Each set of the pots were treated with Jeevamrutha, Sea weed, KNF and General water respectively at 15, 30, 45 and 60 days interval.

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Preparation and application of biostimulants

Jeevamrutha

Jeevamrutha was prepared using standard protocols (Palekar *et al*., 2006) with slight modifications. Jeevamrutha was prepared by mixing 10 kg of cow dung, 10 litre of cow urine, 2 kg of local jaggery, 2 kg of gram flour and handful of cultivated soil in a plastic drum and made up to 200 litre. The solution was regularly stirred clockwise in morning, afternoon and in evening continuously for 10 days and it was used for soil application.

Sea weed

Sea weed manure extraction was prepared with slight modifications (Kumar *et al*. (2012). *Sargassum swatzii* sea weed collected from South East coast regions of Tamil Nadu, India. The collected sea weed samples were washed with sea water followed by fresh water in order to remove sand, macroscopic epiphytes and salt. Samples were kept for 4 days shade dry followed by oven dry at 60°C for 12 hrs. Then the materials were made to coarse powder using mixer grinder. Later the material was added with distilled water in 1:20 (w/v) ratio and autoclaved at 121°C, 15lbs pressure for thirty minutes. The hot extract was filtered through cheese cloth and allowed to cool at room temperature (Rama Rao, 1990).

Korean Natural Farming (KNF)

Korean Natural Farming involves the collecting and culturing of indigenous microorganisms (IMO) (Cho and Cho, 2010; Drake, 2012). The input of IMO includes fish amino acids (FAA), fermented plant juice (FPJ) and fermented fruit juice (FFJ).

Morphological parameters

Morphological parameters such as shoot length (cm), root length (cm), number of leaves, number of branches and number of root nodules were measured with fifteen days interval.

Physiological parameters

Root to shoot ratio (%)

The roots and shoots were dried in a hot air oven at around 80 °C for about 48 hours (till attaining constant weight). The Root to shoot ratio was calculated as per the formula of Anbularmathi *et al.* (2008).

$$\text{Root to Shoot ratio} = \frac{\text{Root dry weight (g)}}{\text{Shoot dry weight}} \times 100$$

Leaf chlorophyll (mg/g)

Total leaf chlorophyll in each treatment was calculated according (Hiscox and Israelstam, 1979). To the 30 mg of leaf material was taken in a test tube, 10 ml of dimethyl sulfoxide (DMSO) was added and the entire set up was kept in hot water bath at 60 °C for about 30 minutes until the chlorophyll pigment was extracted into DMSO. The optical density was recorded at 645 and 663 nm by using UV–VIS spectrophotometer (Elico *SL 159*). The amount of chlorophyll pigment present in the sample was calculated by using the following formula.

$$\text{Total chlorophyll} = (20.2 \times \text{O.D at 645 nm} + 8.02 \times \text{O.D at 663 nm}) \quad \frac{V}{10} \times W$$

Total leaf area per plant (cm2)

The leaves were collected and kept in polythene bags to avoid wilting and immediately brought to the lab. The total leaf area per plant was measured by using *LICOR 3100* leaf area meter.

Yield parameters

Yield parameters such as pods per plant, seeds per pod, pod length, whole plant dry weight (g), 100 seed weight (g) and yield/plant (g) were measured at the harvest.

Statistical analysis

Data was analyzed using analysis of variance (ANOVA) following randomized block design (Gomez and Gomez, 1984). Differences were considered significant at 5% level of probability.

The present study showed significant morphological change in *Vigna unguiculata* L. Walp (Plate 1). The effect of all the biostimulants on the shoot growth were examined at 15, 30, 45 and 60 DAS. The shoot length was ranged between 16 cm to 62 cm with a mean shoot length of 19.7 to 55.7 cm. Of all the biostimulants maximum shoot growth was observed in KNF treatment at 15, 30, 45 and 60 DAS. Plant treated with KNF showed highest shoot length (62.0 cm), followed by Jeevamrutha (60 cm) at 60 DAS (Table 1) which is quiet higher than the control plants. Plants treated with various biostimulants reported varied root length ranged between 7 to 20 cm with a mean root length of 9.5 to 17.7 cm. KNF treated plants exhibited higher root length 20 cm at 60 DAS followed by Jeevamrutha (17 cm) and sea weed (19 cm) whereas, the untreated plant reported decreased root length (15 cm) (Table 1). Leaf number was ranged between 16 to 44 with a mean of 25.7 to 39.7 (Table 1). Among all the biostimulants plant treated with Jeevamrutha showed more leaf number 8, 19, 31 and 44 at 15, 30, 45 and 60 DAS respectively. After Jeevamrutha KNF (41) and sea weed (40) treated plant reported higher leaf number at 60 DAS. Growth of branches due to biostimulant treatment was observed and tabulated in Table 1. Number of branches per plant was varied between 2 to 12 with a mean value of 5 to

Table 1. Effect of various biostimulants on morphological characters of *Vigna unguiculata* (L.) Walp

Parameter	Shoot length(cm)				Root length(cm)				Number of leaves				Number of branches			
No. of Days→ Treatments↓	15	30	45	60	15	30	45	60	15	30	45	60	15	30	45	60
Un-treated	16	25	36	48	7	10	11	15	6	16	27	34	2	4	7	9
Seaweed	18	29	40	53	9	12	13	19	10	18	29	40	2	5	9	10
Jeevamrutha	22	32	46	60	10	13	15	17	8	19	31	44	3	7	10	12
KNF	23	34	48	62	12	15	17	20	7	18	32	41	3	5	9	11
Grand mean	19.7	30	42.5	55.7	9.5	12.5	14	17.7	25.7	17.7	29.7	39.7	5.0	5.2	8.7	10.5
SEM±	3.3	3.9	5.5	6.4	2.08	2.08	2.58	2.21	1.70	1.25	2.21	4.19	0.57	1.25	1.25	1.2
CV	2.35	2.8	3.20	3.50	2.54	2.58	2.98	3.54	1.52	1.85	2.00	2.14	3.54	4.32	5.55	6.21

*Significant at 5%

10.5. The mean number of branches recorded more in Jeevamrutha treated plants (12) when compared with other treatments KNF (11), sea weed (10) and control (9) plants. Number of nodules per plant was increased up to 60 DAS (Fig. 1A). The mean root nodule number was ranged between 44.5 to 90.5. Of all the treatments maximum production of root nodules 120 were observed in KNF treated plants followed by Jeevamrutha and sea weed treated plants whereas, the untreated plants produced less number of root nodules. Root to shoot ratio recorded and showed in Fig. 1B. In present study highest root to shoot ratio was reported in KNF treated plants at 15, 30, 45 and 60 DAS. Leaf chlorophyll content was found to be increased in varied treatments (Fig. 1C). Highest leaf chlorophyll content was reported with the plants treated with KNF at 60 DAS (1.92 mg/g) followed by Jeevamrutha (1.84 mg/g), Sea weed (1.79 mg/g) and 1.48 mg/g in untreated plants. Total leaf area per plant was recorded and was shown in Fig. 1D. In present study highest leaf area per plant was observed in plants treated with KNF at 60 DAS (332.25 cm^2) followed by Jeevamrutha (287.31 cm^2), Sea weed (271.58 cm^2) and untreated plants (195.11 cm^2).

Impact of various biofertilizers such as sea weed, jeevamrutha and KNF on yield attributes was studied and analyzed. The results recorded significant differences among the various biofertilizers treatments both at 45 DAS and 60 DAS. The number of pods per plant was ranged from 2-8 at 45 DAS and 12 to 22 at 60 DAS. Both at 45 and 60 DAS plants treated with KNF showed a greater number of pods per plant (8 and 22) when compared to other treatments (Table 2). Seeds per pod was recorded and tabulated and it was varied between 4 to 19 with an average mean of 6.25 and 13.5 at 45 DAS and 60 DAS. Of all the treatments studied KNF treated plant reported highest number of seeds per pod both at 45 DAS (8) and 60 DAS (19) followed by jeevamrutha and sea weed treatments. Increased pod length was observed in treated plants when compared to the control plants (Table 2; Plate 2). Significant pod length was recorded in present study in all the treatments and it was ranged from 22.3 cm to 35.2 cm with a mean of 27.42 and 30.25 both at 45 DAS and 60 DAS. The highest pod length of 31.4 cm and 35.2 cm was reported in KNF treated plants both at 45 DAS and 60 DAS respectively. Whole plant dry weight was found

Table 2. Effect of various biostimulants on yield parameters of *Vigna unguiculata* (L.) Walp

Parameter	Pods / plant		Seeds /pod		Pod Length (cm)		Whole Plant Dry Weight (g)		100 Seed weight (g)	Yield/plant (g)
No. of Days→ Treatments↓	45	60	45	60	45	60	45	60		
Un-treated	2	12	4	8	22.3	25.0	7.82	9.75	10	2.54
Seaweed	5	16	6	12	25.6	29.5	9.10	11.27	15	3.14
Jeevamrutha	6	17	7	15	30.4	31.3	10.11	12.87	16	3.98
KNF	8	22	8	19	31.4	35.2	12.18	14.98	23	4.55
Grand mean	5.25	16.75	6.25	13.5	27.42	30.25	9.802	12.21	16.0	3.55
SEM±	0.87	0.99	0.59	0.98	2.06	2.58	1.7	2.10	1.24	0.61
CV	5.24	6.35	3.58	5.33	4.21	5.64	3.58	4.10	2.35	2.99

*Significant at 5%

to be increased in various treatments (Table 2). It was ranged from 7.82 to 14.98 with an average mean value of 9.80 g and 12.21 g at 45 DAS and 60 DAS. And maximum dry weight was recorded with KNF treated plants (12.18 g and 14.98 g at 45 DAS and 60 DAS). The 100 seed weight was measured and it was ranged between 10 g to 23 g with a mean value of 16.0 g (Table 2). Highest seed weight of 23 g was reported with KNF treated plants followed by Jeevamrutha (16 g), Sea weed (15 g) and untreated plants (10 g). The total harvest of the plants under various biofertilizer treatments was measured and tabulated (Table 2). Significant change in yield with varied treatment was observed. The yield per plant under different biostimulant treatments was ranged from 2.54 g to 4.55 g with a mean value of 3.55 g. Of all the treatments maximum crop yield per plant was recorded when plants treated with KNF (4.55 g) followed by jeevamrutha (3.98 g), sea weed (3.98 g) and control plants (2.54 g).

Annually about 175.5 million tons of chemical fertilizers were used world over to get the optimum yields (FAO, 2011). Extensive use of chemical fertilizers led to the contamination of both water bodies and cultivated lands. In present study significant increase in growth parameters was observed when treated with different biostimulants. The mean performance of all morphological characters of cowpea (Khasi kanchan) was tabulated in Table 1. The shoot length has been recorded highest in KNF treated plants (62 cm) whereas the lowest have been recorded in the untreated plants (48 cm). The higher shoot length is may be due to aminoacids and beneficial microorganisms present in the KNF (Maghirang, 2011; Sherri *et al*., 2013). The maximum root length is recorded with KNF treated plants (20 cm) subsequently followed by Seaweed. The number of leaves shows close proximity in the initial days but as the days are increasing the highest number of leaves is recorded in Jeevamrutha, followed by KNF and Sea weed. Moreover, the number of branches was recorded highest in Jeevamrutha (Rouphael, *et al.,* 2018a). Our findings are in agreement with that of Reshma *et al*. (2018) in having highest number of branches and number of leaves with the application of jeevamrutha in cowpea. Increase in growth attributes due to jeevamrutha application might be attributed to solubilisation of soil nutrients coupled with absorption of nutrients and moisture (Siddappa *et al*., 2016). Similar trend of results were observed in cowpea by Sivasankari *et al*. (2006).

The impact of biostimulants on yield characteristics was tabulated in Table 2. The highest number of the pods has been recorded in KNF treated cowpea plants. Seaweed and jeevamrutha shows a close proximity in having equal number of pods. The number of seeds per pod (19), length of the pod (35.2 cm) and per plant yield (4.55 g) is significantly highest in KNF treated plants. Enhanced growth in cowpea on application of biostimulants is due to improved favorable conditions for the luxuriant growth of soil rhizobia and IMO (Sherri *et al*., 2013). Increased soil microorganisms have helped translocation of carbohydrates to developing root nodules (Sait and Kibritci, 2016). On the other side KNF enriched with growth hormones IAA, GA3 promotes the microbial population (Cho, 2010). The Korean Natural Farming is a great alternative for the farms looking to become self-sufficient.

KNF is the rich source of the beneficial microorganisms such as nitrogen fixing and phosphate solubilizing bacteria. From this study, it is found that KNF is effectively improved the efficient microbial consortia thereby increasing NPK content and plant growth promoting factors. KNF is the best substitute to chemical fertilizer to improve soil fertility thereby crop productivity and quality.

Authors' contribution

Conceptualization of research work and designing of experiments (GVS, VR); Execution of field/lab experiments and data collection (GVS, VR); Analysis of data and interpretation (KB); Preparation of manuscript (GVS, KB, VR)

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