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## REGULAR ARTICLE

## EFFECT OF BIO-STIMULATORS ON GROWTH AND YIELD OF COWPEA LEAVES (*VIGNA UNGUICULATA* WALP) PRODUCED IN JUJA AND KATUMANI IN KENYA

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**ABSTRACT**

Bio-stimulators are organic products that have been found to enhance crop growth and productivity through mitigating against biotic and abiotic stresses. Four Cowpea varieties namely K80, M66, KVVU 27-1 and Kenya Kunde; commercially released and adopted by local households for vegetable production were used to evaluate the impact of bio-stimulators on growth and leaf yield of cowpea in Juja and Katumani from May to July, 2013. The treatments were arranged in a factorial structure, laid out in an RCBD, which were combination of 9 levels of bio-stimulators, with the 4 cowpea varieties planted per site, and giving a total of 36 treatments for each site replicated 3 times. The plots measured 2.5m by 2.5m with spacing of 60 cm by 20 cm. Parameters under investigation included germination rate, days to germination, plant height, and number of leaves produced and number of nodules formed. Cultural practices such as weeding and pest control were done as need arose. The results showed that there were significant differences in days to germination, germination rate, plant population, chlorophyll levels, leaf weight, plant height and leaf area but not in leaf area between Juja and Katumani. Earliest germination and highest leaf weight were in Katumani whereas highest plant population, germination rate and chlorophyll levels and leaf area were in Juja. Humates improved germination rate, plant height, leaf weight and nodule development in Juja while seaweed extracts improved days to germination, germination rate, plant height, leaf yield, leaf weight and nodule formation in Katumani. Interaction between site and bio-stimulators was recorded in germination rate, days to germination, plant population and in number of leaves produced. Therefore, bio-stimulators should be adopted in production of cowpea leaves particularly off-season for food and nutritional security and increasing household incomes in Kenya

**Keywords:** Humates, seaweed extracts, biotic and abiotic stresses, nodule, local households, food security

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**INTRODUCTION**

Bio-stimulators are natural substances that stimulate plant processes at very low concentrations. They are derived from plants and animals and contain amino acids, low molecular weight polypeptides, vitamins, enzymes, hormones (cytokinins, auxins and gibberellins), sugars, betaines and antioxidants (1,2). They are either single or multi-ingredient preparations supporting biochemical processes therefore enhancing plants' resistance to biotic and abiotic stresses upon application without interfering with the inherent plant processes and pathways (3). Bio-stimulators have been found to influence plant processes

such as photosynthesis, respiration and nutrient availability (5,6), inhibit heavy metals uptake by plants (1) and improve plant tolerance to pests through invoking a systemic acquired resistance correlated with accumulation of parthenogenesis related proteins (PR) (3).

Studies have shown that bio-stimulators applied on different crops have positively influenced crop growth thereby leading to better yields. Bio-stimulators sprayed on tomato plants in vegetative stage increased fruit yield by 30% and the number of flowers and seeds per flower head in Marigold seedlings by 50% (7). Other bio-stimulators such as Maxi crop® (*Ascochyllum nodosum*)

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and (*Ecklonia maxima*) enhanced yields in lettuce, barley pepper and beans by 24% (7). Application of bio-stimulators to a cabbage crop led to increased plant growth and activity of microbes antagonistic to *Pythium ultimum* that causes damping-off disease and pests (aphids) generally avoided plants treated with seaweed extracts (8). In Kenya use of bio-stimulators in potato production led to 15% increase in marketable tuber yield in the high potential areas (9). Use of bio-stimulators in Kenya has been done though on a small scale and only in high potential areas, despite the fact that they have been found to be more effective in abiotic and biotic stressed environments. Cowpea is a popular traditional vegetable crop in Kenya whose production is exponentially rising, hence chosen as a model crop. Cowpea is also a popular leafy food legume with high source of dietary protein and nutritious fodder in the semi-arid tropics (10). Cowpea seeds contain 21-33% proteins while the leaves contain 29-43% vegetable proteins on a dry weight basis thus provides protein to majority of the rural and urban poor population that cannot afford animal proteins, hence a potential crop for attaining nutritional security (11). Bio-stimulators have been found globally to have great potential in enhancing crop productivity especially vegetable production but little work has been done with them in Kenya on crop production in harsh environments such as drought, hence forming the basis of this study.

**MATERIALS AND METHODS**

**Experimental site**

The study was conducted in Katumani, Machakos County and Jomo Kenyatta university of Agriculture and Technology (JKUAT) in Kiambu County from May to July 2013. JKUAT lies at an altitude of 1525m above sea level and latitude of 1° 10' 48' S, long. 37° 07' 12' E and is located in the LH 2. Rainfall amount in this area ranges from 600-800 mm annually with average temperatures of 25 °C. (12). Katumani lies at an altitude of 1575 m above sea level and latitude of 1° 35' S and 37° 14' E in agro-ecological zone IV. Rainfall amount in the area ranges from 500-700 mm per year with mean 4 commercially released and locally adopted

cowpea varieties identified for leaf and grain production were selected namely K80, M66, KVVU 27-1 and Kenya Kunde. K80, M66 and KVVU 27-1 were obtained from the Basic Seed Unit of Kenya Agricultural and Livestock Research Organization (KALRO) Katumani while Kenya Kunde was bought from Agrochemical shop.

**Test crop**

**Experimental design**

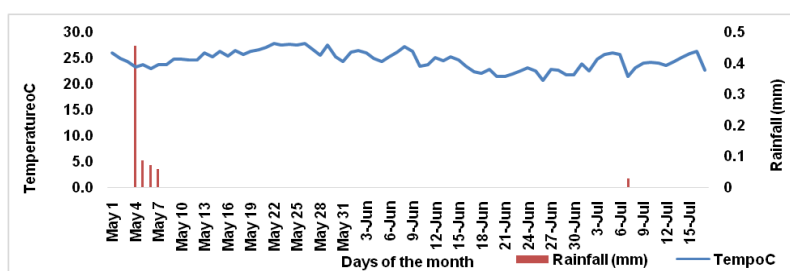
Land was ploughed and harrowed and plots measuring 2.5m by 2.5 m were prepared. Planting was done at a spacing of 60 cm by 20 cm giving a population of 96,000plants/ha (13). The layout was a combination of the 4cowpea varieties, planted in 2 sites and the 9 levels of bio-stimulators giving a total of 36 treatments for each replicate; replicated thrice. Bio-stimulators were applied at planting except for foliar sprays which were done from 3 leaf stage up to flowering stage. All cultural practices were done as need arose (13).

**Data analysis**

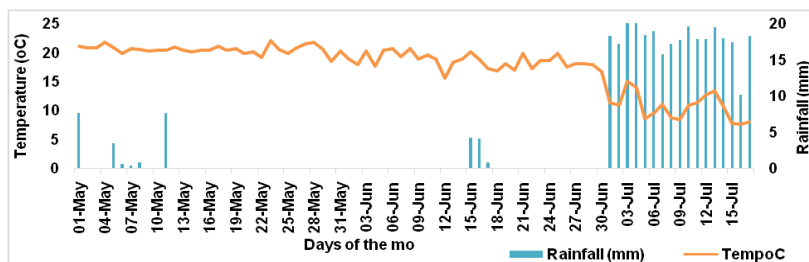
Data was analyzed using GenStat statistical package 12th edition to determine interaction between treatments (14). Treatments were analysed using ANOVA while Separation of means was done by the Fisher's protected Least Significant Difference (LSD) test at 5% confidence interval.

**RESULTS**

The results showed that there were significant differences between Juja and Katumani in germination rate, days to germination, plant count, chlorophyll levels, plant height, leaf weight, leaf yield and number of nodules formed but not in stem thickness, leaf area or root length at P<0.05 (table 1; Figs 2 and 3). Days to germination, rate of germination, survival rate, chlorophyll levels, leaf weight, and leaf area were higher in Juja than in Katumani (table 1). Plant height was also higher in Juja than in Katumani from week 1 to 8 (fig. 2)



**Fig. 1a: Rainfall and temperature in katumani from may to july**

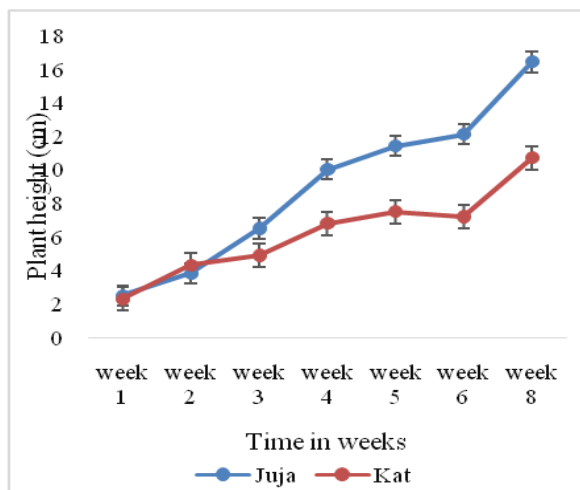


**Fig. 1b: Rainfall and temperature in juja from may to july**

**Table 1: Performance of parameters between Juja and Katumani**

Site	Days to germination	Rate of germination %	Survival rate	Chlorophyll levels	Leaf weight (g)	Leaf area (cm <sup>2</sup> )
Juja	8 <sup>b</sup>	60 <sup>a</sup>	70.4 <sup>a</sup>	54.4 <sup>a</sup>	1.6 <sup>b</sup>	39.2 <sup>a</sup>
Katumani	5 <sup>a</sup>	29.2 <sup>b</sup>	34.4 <sup>b</sup>	48.9 <sup>b</sup>	2.2 <sup>a</sup>	34.5 <sup>a</sup>
Mean	6	45	52.4	51.6	1.9	36.8
LSD	0.8	4.9	6.1	2.7	0.2	5.1
P	<.001	<.001	<.001	<.001	<.001	0.07

Means followed by the same letter are not significantly different at P≤0.05



**Fig. 2: Plant height in Juja and Katumani**

Bio-stimulators were significantly different between Juja and Katumani in germination rate, days to germination, plant count, leaf area, leaf yield and nodulation but not in chlorophyll levels, plant height, stem thickness and leaf weight at P≤0.05 (table 2). Juja had the highest rate of germination, survival rate, leaf area, leaf weight and chlorophyll levels (tables 2-3). Earliest germination was in Katumani. SWE basal application caused earliest germination in both sites, although both bio-stimulators caused early germination also.

**Days to germination:** SWE caused early germination of 5 d as compared to control and humates (11 d) in Juja while in Katumani, in 4 d.

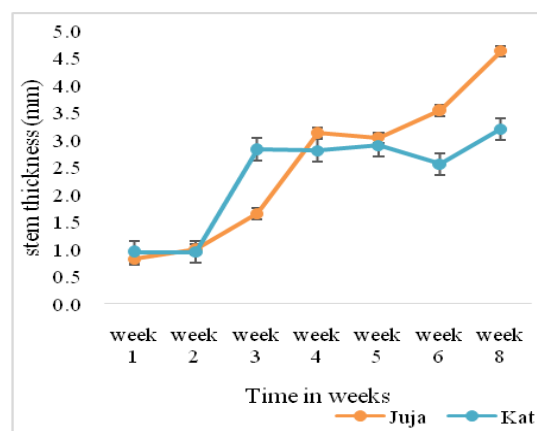
**Germination rate:** Highest germination rate was in humates in Juja and SWE basal application and foliar in Katumani (table 2).

**Plant count:** Juja had the highest number of plants established of 70±6.7 compared to Katumani with 34±7.0. Humates had the highest number of plants in Juja of 81±5.6 while SWE basal application and foliar spray in Katumani with 72±6.6 (table 3).

**Leaf area:** The highest leaf area was recorded in humates with 50.7±4.4 cm<sup>2</sup> in Juja while in Katumani 54.1±1.8.

**Leaf weight:** The highest leaf weight was recorded in humates (Katumani) with 2.5±0.3g (table 3).

**Chlorophyll:** Chlorophyll levels were highest in humates with SWE foliar spray in Juja (56.6±1.1) while in Katumani it was in SWE basal application with 54.1±1.8.



**Fig. 3: Stem thickness in Juja and Katumani**

**Table 2: Germination rate, days to germination and plant count in bio-stimulators**

Treatment	Rate of germination		Days to germination		Plant count	
	Juja	Katumani	Juja	Katumani	Juja	Katumani
Humates	67±4.6	15±5.3	11±0.19	4±0.44	81±5.6	17±6.5
Humates+SWE basal application	60±5.1	37±8.2	5±0.27	4±0.40	70±7.0	43±9.4
Humates+SWE basal application+SWE foliar	56±5.5	40±4.8	5±0.36	4±0.00	61±8.3	47±5.4
Humates+SWE foliar	64±4.8	15±7.1	11±0.26	6±0.86	76±5.8	17±8.2
SWE basal application	61±5.5	44±6.6	5±0.26	4±0.00	72±6.4	52±7.1
SWE basal application+SWE foliar	51±4.8	60±5.6	5±0.37	4±0.08	60±6.9	72±6.6
SWE foliar	63±4.4	23±6.1	11±0.36	5±0.27	74±6.4	27±7.3
Biofix	62±5.1	3±1.6	10±0.19	6±0.94	70±7.6	4±1.9
Control	59±5.2	27±8.6	11±0.19	7±1.50	71±6.2	32±10.3
Mean	61±5.2	29±8.6	8±0.29	4±3.75	70±6.7	34±7.0
P	<.001***		<.001***		<.001***	

Means \*\*-highly significant, \*-significant, NS-not significant at P≤0.05; with SE separation at P<0.05

**Table 3: Leaf weight, leaf area and chlorophyll levels between bio-stimulators**

Treatments	leaf weight (g)		leaf area(cm <sup>2</sup> )		Chlorophyll levels	
	Juja	Katumani	Juja	Katumani	Juja	Katumani
Humates	2.1±0.2	2.4±0.5	49.4±4.7	42.6±5.6	54.0±1.3	41.6±1.3
Humates+SWE basal application	1.4±0.2	2.4±0.3	33.7±4.8	25.8±5.2	52.2±1.6	51.3±4.8
Humates+SWE basal application+SWE foliar	1.5±0.2	2.1±0.3	40.0±6.1	34.4±5.5	54.1±1.1	51.7±2.4
Humates+SWE foliar	2.0±0.2	2.1±0.4	50.7±4.4	40.6±3.3	56.6±1.1	46.9±0.9
SWE basal application	1.4±0.2	2.4±0.3	36.7±5.2	33.8±6.6	55.3±1.3	54.1±1.8
SWE basal application+SWE foliar	1.5±0.3	2.5±0.3	35.8±8.0	41.9±9.4	55.1±1.1	53.5±1.3
SWE foliar	1.7±0.2	2.1±0.2	39.9±5.7	37.9±6.1	53.6±1.1	43.3±2.3
Biofix	1.3±0.2	2.0±0.3	32.1±4.9	24.2±4.1	53.8±0.7	43.5±0.9
Control	1.5±1.0	1.9±1.0	34.4±3.5	29.2±4.9	54.9±1.2	50.8±1.9
Mean	1.6±0.1	2.1±0.1	34.5±1.8	29.2±2.0	54.4±0.8	48.9±1.4
LSD	0.7		15.9		8.0	

\*\*-highly significant, \*-significant, NS-not significant at  $P \leq 0.05$ ; with SE separation at  $P < 0.05$

**Nodules:** Bio-stimulators influenced nodulation from weeks 5 to 8 in Juja but in Katumani only up to week 6. Highest number of nodules were recorded in

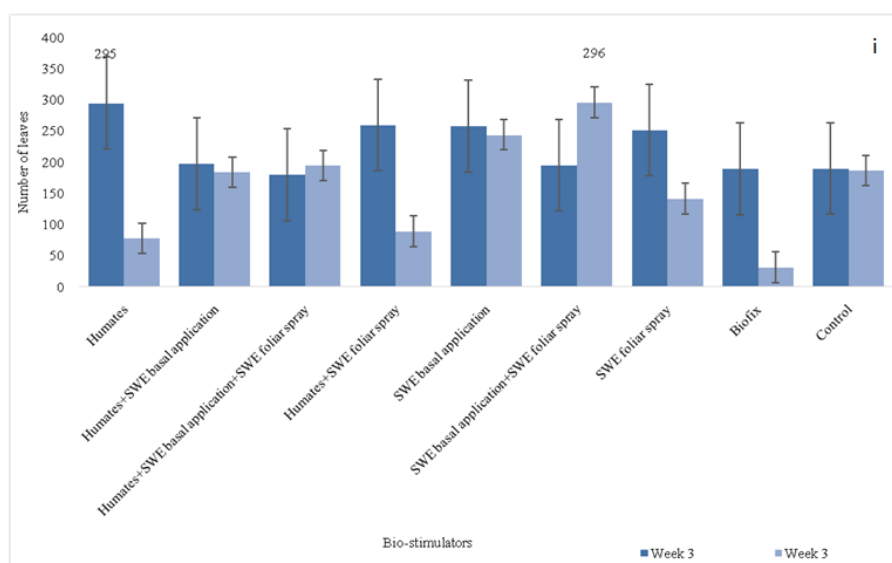
**Table 4: Nodulation in bio-stimulators between Juja and Katumani**

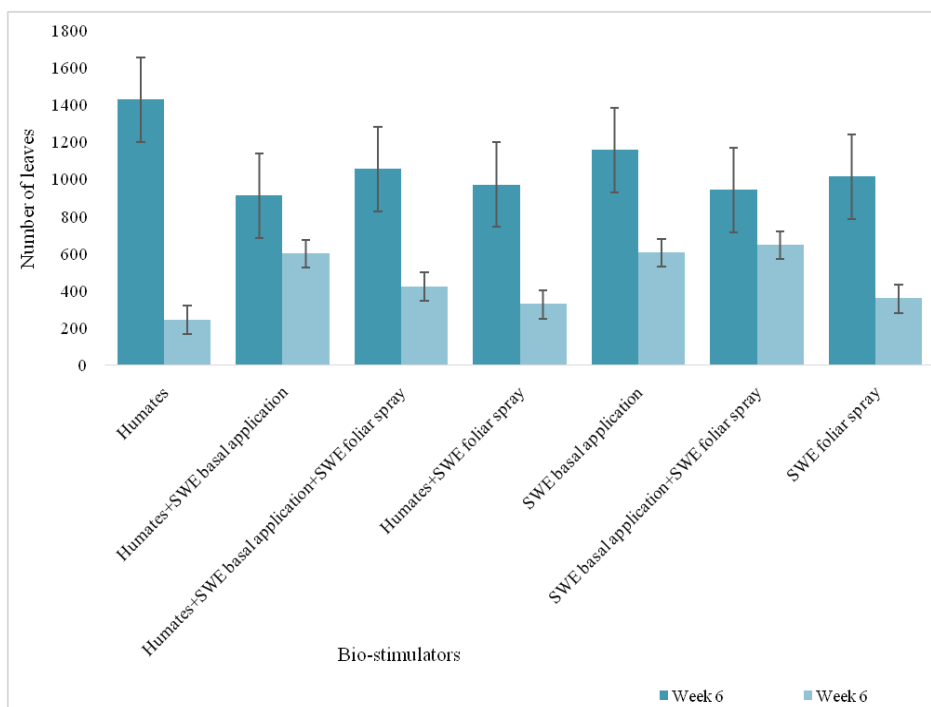
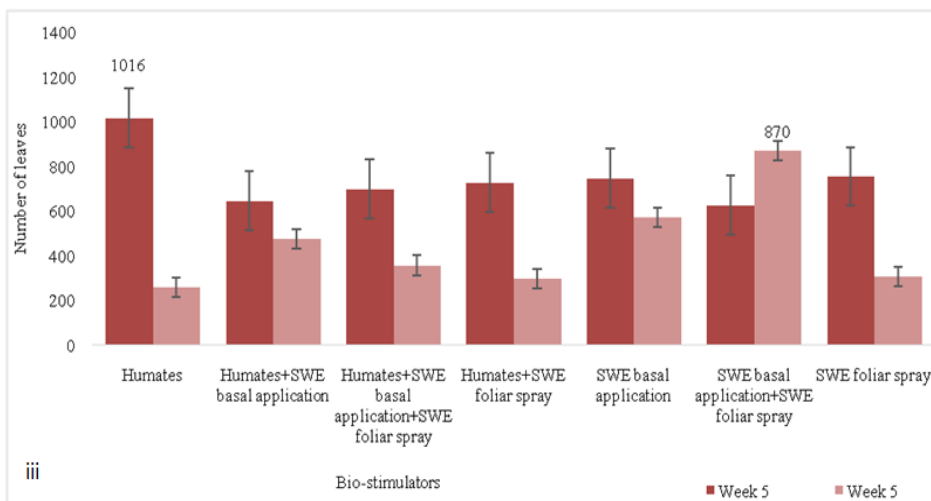
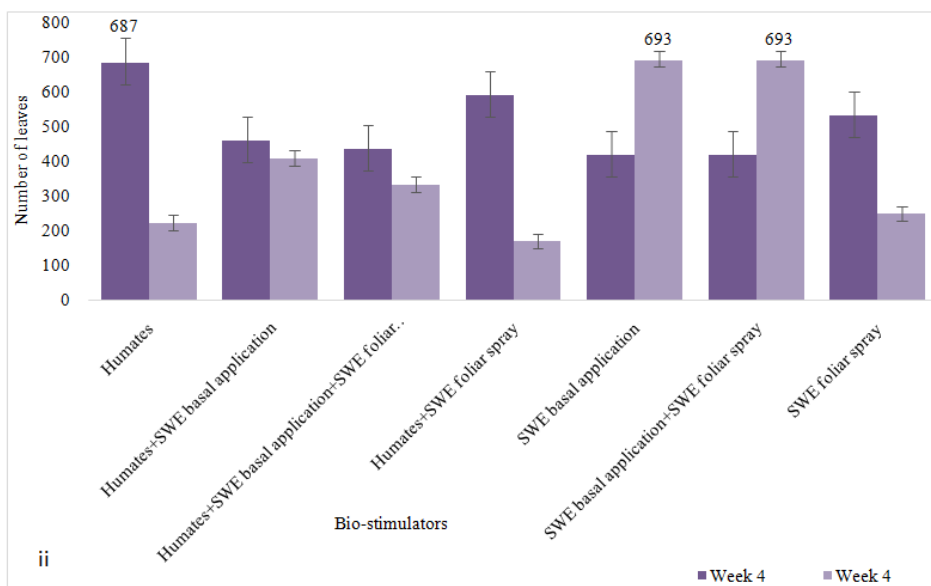
	Week 5		Week 6		Week 8	
	Juja	Katumani	Juja	Katumani	Juja	Katumani
Humates	6a	6ab	9a	2ab	21ab	11a
Humates+SWEbasal application	5a	7ab	11a	3ab	10bc	3a
Humates+SWE basal application+SWE foliar spray	6a	11a	8a	3ab	18abc	2a
Humates+SWE foliar spray	4a	6ab	9a	1b	19abc	4a
SWE basal application	6a	9ab	9a	6a	17abc	2a
SWE basal application+SWEfoliar spray	8a	12a	9a	4ab	8c	3a
SWE foliar	5a	5ab	9a	2ab	12abc	6a
Biofix	4a	2b	9a	1b	11abc	7a
Control	5a	7ab	8a	3ab	22a	10a
Mean	5.4	7.2	9	2.7	15.3	5.3
Lsd	6		5		6	
P value	0.04*		<.001**		<.001**	

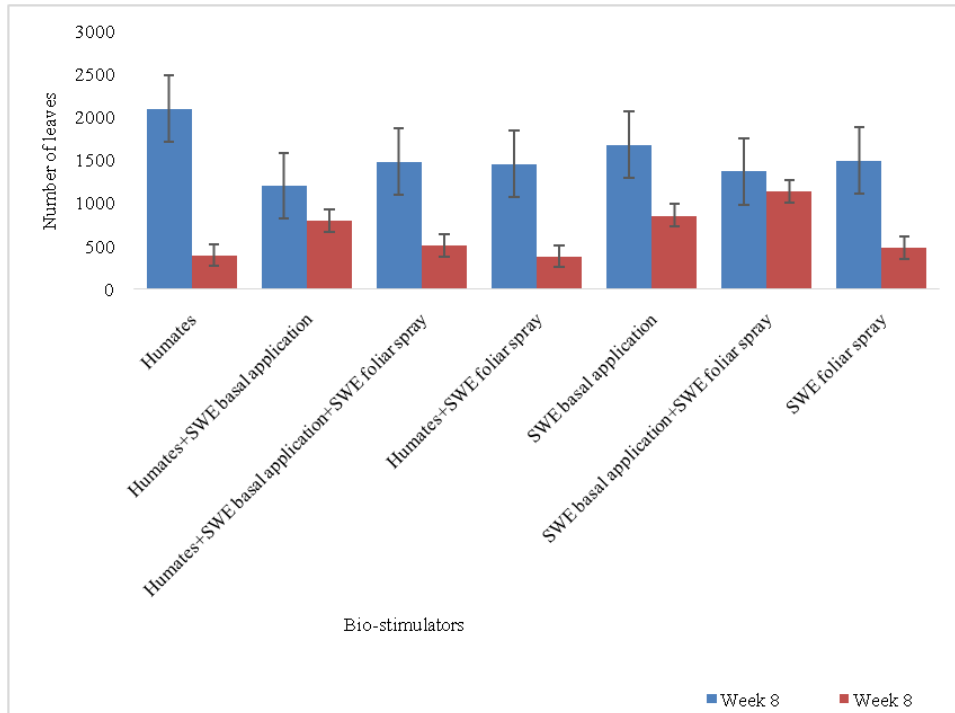
\*\*-highly significant, \*-significant, NS-not significant at  $P \leq 0.05$ ; Means followed by the same letter are not significantly different at  $P \leq 0.05$

**Leaf yield:** Production of leaves was highest in Juja mainly in humates from week 3 to 8. Katumani had the highest number of leaves produced in SWE basal

application with foliar spray (fig. 3i-iii). Katumani produced the highest number of leaves in week 3, though by a leaf (fig. 3i)





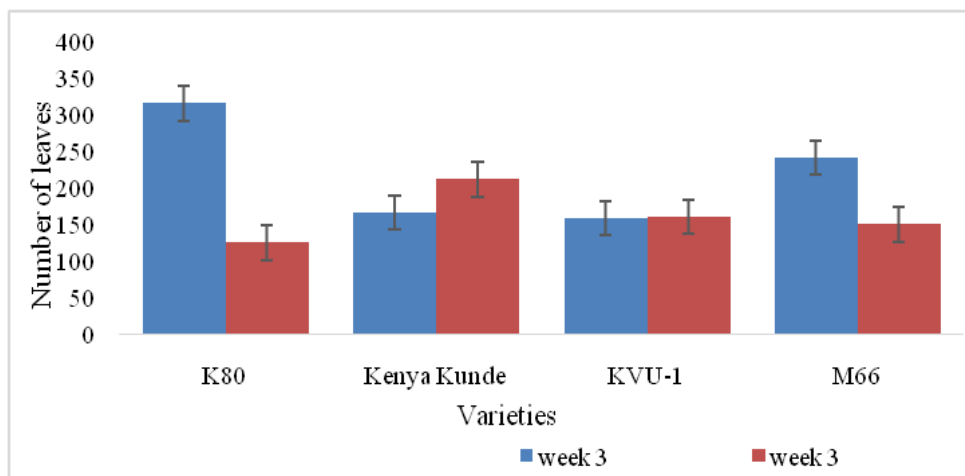


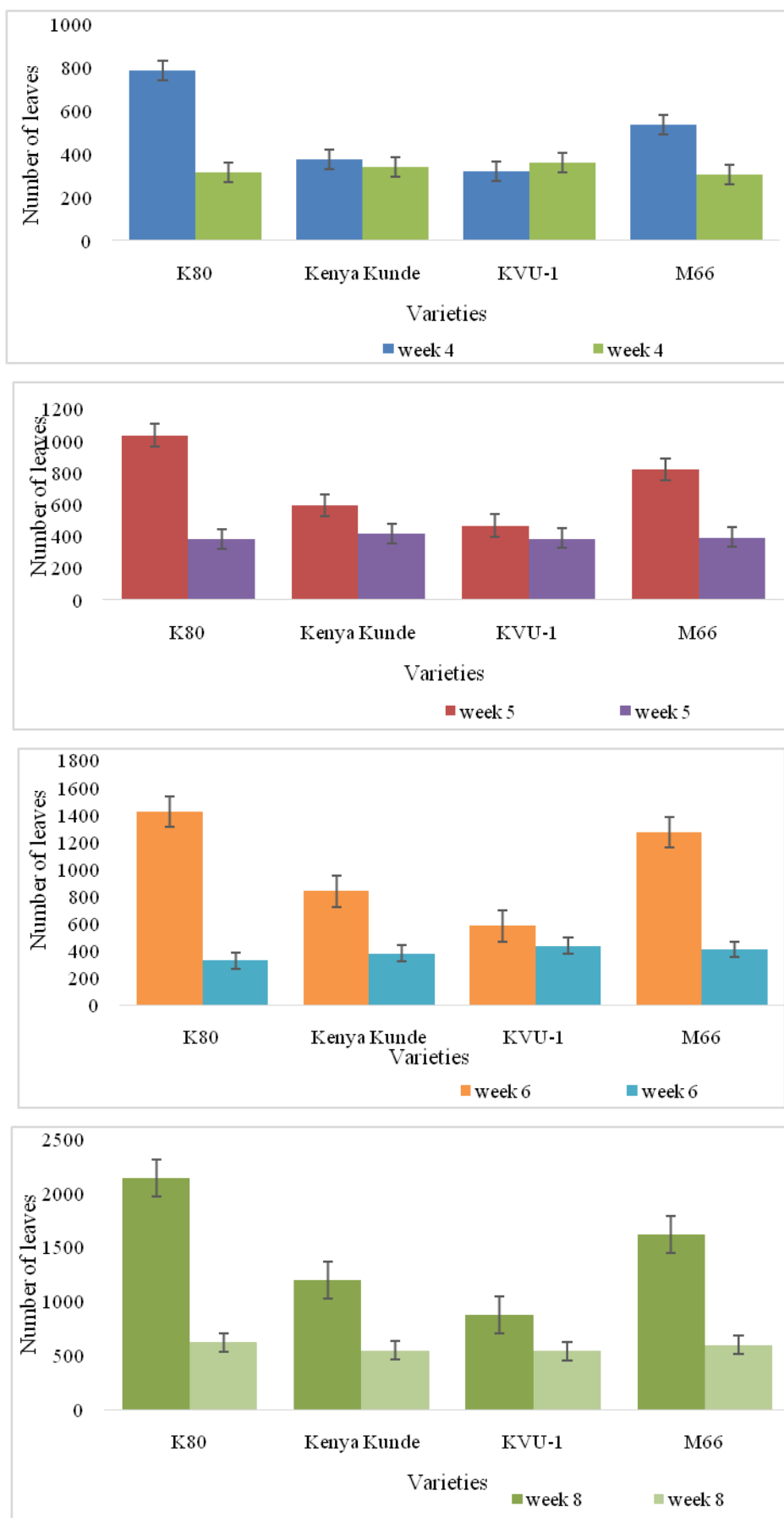
**Fig. 4: Number of leaves produced in bio-stimulators from week 3(i) to week 8 (v), SE of means at  $P \leq 0.05$ , Varieties were significantly different between Juja and Katumani in germination rate, plant count and number of leaves produced at  $P \leq 0.05$  (table 4)**

**Table 4: Performance of varieties between Juja and Katumani**

Varieties	Week 3	Week 4	Week 5	Week 6	Week 8
K80	220	549	712	894	1378
Kenya Kunde	189	356	511	607	870
KVU 27-1	167	341	431	509	717
M66	195	420	609	840	1106
Mean	193	416	566	713	1018
LSD	47.8	93.6	141	181	280
P	<.001***	<.001***	<.001***	<.001***	<.001***

\*\* ,\*\*\*-highly significant, \*-significant, NS-not significant at  $P \leq 0.05$ ; with SE separation at  $P < 0.05$





**Fig. 5:** Number of leaves produced in Varieties from week 3(i) to week 8 (v), *SE of means at  $P \leq 0.05$*

## DISCUSSION

The results described in this study showed that bio-stimulators which are humates and seaweed extracts (SWE) of *Ecklonia maxima* improved cowpea growth, leaf production and leaf weight (7). The humates and seaweed extracts applied at planting led to early germination and higher germination rates by between 9-52% in all the varieties (table 1b) agreeing with findings of some researchers (7) that bio-stimulators applied at an early stage tended to enhance germination and plant growth. Some studies done proved that potassium humates and soluble potash present in seaweed extracts caused early germination and increased germination rate of between 37-64% in Okra (25,15). Plant height (fig. 2), leaf weight and leaf yield (Tables 2 and 3) were observed to be higher in Juja in humates while in Katumani in SWE treatments. Humates and SWE have been found to contain substantial quantities of cytokinins, carbohydrates, amino acids, vitamins, minerals, micro nutritive elements, betains and anti-oxidants such as phenolic acids and flavonoids which upon application exogenously either during planting as drench or foliar spray triggered higher levels of endogenous cytokinins and auxins that led to early germination, higher root and shoot growth, nutrient mobilization, increase in root mass, photochemical efficiency, anti-oxidant levels and anti-senescence properties of various turf grass species during abiotic stresses (8, 16; 2; 17;18). Studies done previously showed 20% seaweed extracts of *Rosenivigea intricata* increased the length and weight of *Cyamopsis tetragonoloba* and watermelon compared to controls (19, 20 and 21). Similar observations were made in studies in *Cajanus cajan*, *Vigna radiate* and *Dolichos biflorus*, potatoes (*Solanum tuberosum*) (9) and *Diptychandra aurantiaca* (22) where application of bio-stimulators at very low concentrations led to higher germination and growth compared to controls (23). Foliar application of SWE increased length, diameter and fruit yields as well as early maturity in pepper (8), cucumber (2) and tomatoes (24) because of rapid absorption of nutrients which are directly and quickly incorporated into the plant metabolism (25,6). Humates have been found to trigger greater root mass as a result of direct contact with the roots leading to initiation of root hypocotyl segments in legumes (7). Further studies have shown that drought-stressed plant treated with both humic acids and seaweed extract increased root mass by 21-68%, foliar tocopherol by 110% and endogenous zeatinriboside by 38% (2;18). Bio-stimulators influenced nodulation in weeks 5 and 6 in Juja and weeks 5 to 7 in Katumani, with the most number of nodules recorded in untreated in week 7 with 22 (table 3). Biosynthesis of auxins and cytokinins has been found to contribute significantly to bacterial growth in the rhizosphere as a result of rapid cell division and nitrogen fixing nodule formation, rapid growth of primary roots and branching (18, 27). Therefore, application of bio-stimulators could have promoted biosynthesis of cytokinins and auxins that could have led to more nodules formed. Due to rainfall failure and hence insufficient moisture in Katumani compared to Juja as observed from the weather report (fig. 1a and b), nodules formed were more in Juja. Nodulation is an energy intensive process requiring sufficient nutrients and moisture for the rhizobia to survive in the soil until they infect the roots, hence insufficient moisture could have been responsible for low number of nodules in Katumani probably due to low survival rate of *Rhizobia* in the soil (7). Similar results were found by Royarath *et al.*

(26) with extracts of *A. nodosum* on *Arabidopsis* and *R. irrigata* on *Abelmoschus esculentus*. Biofix as a commercial bio-stimulator did not have great impact probably also due to harsh environmental conditions for any significant impact to be recorded.

## CONCLUSION

Bio-stimulators have the potential to enhance germination and establishment, increase crop growth vigour and eventually improve cowpea leaf yields. Humates performed best in Juja while in Katumani the seaweed extracts (SWE). K80 produced more foliage than all the other varieties.

## RECOMMENDATION

Bio-stimulators should be used to grow cowpea in environmentally harsh conditions for increased food security and wealth creation through sale of surplus.

## ACKNOWLEDGEMENT

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