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Combined Effect of Effective Microorganisms and Seaweed Concentrate Kelpak® on Growth and Yield of Cabbage

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Abstract

The aim of this study was to determine the combined effect of Effective Microorganisms (EM) and seaweed concentrate (SWC) Kelpak® on growth and yield of cabbage under shade house and micro-plot, which is currently not documented. Treatments comprised control, Kelpak®, EM and EM+Kelpak® arranged in RCBD with 10 replicates. Number of leaves and seedling height were taken bi-weekly from 2nd week after transplanting until the 6th week. Four-month after transplanting, chlorophyll content, weight of entire plant, fresh leaf, fresh head, fresh root, dry leaf, dry head and dry root, polar and equatorial diameter of head, head shape and stem diameter were also measured. Combination of EM and Kelpak® significantly ($P \leq 0.05$) increased number of leaves and improved seedling height at week 6 with the exception of week 2 and 4 under both sites. Results further showed that under both sites, EM+Kelpak® improved chlorophyll content, and increased weight of entire plant, fresh leaf, fresh head, and stem diameter. Under shade house, EM+Kelpak® improved head polar diameter. However, under both sites, treatments were not significant on the weight of fresh root, dry root, dry leaf, dry head and equatorial head diameter. In conclusion, combination of EM and Kelpak® improved cabbage growth and yield under both sites.

Keywords: Bio-stimulants, kelpak®, effective microorganisms, seaweed concentrates.

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Introduction

The Effective Microorganisms (EM) and seaweed concentrates (SWCs) bio-stimulants were both introduced in 1950s and 1989, respectively, with the focus of eliminating environmental problems associated with the use of agrochemicals in agriculture (Milton, 1952; Javaid and Bajwa, 2014). Several studies have been carried out to demonstrate that the two bio-stimulants were eco-friendly and safe agricultural inputs (Hu and Qi, 2013; Vijayand *et al.*, 2014). In the last two decades, these bio-stimulants had been thoroughly researched either singly or in combination with other agrochemical and organic inputs (Khaliq *et al.*, 2006; Chouliaras *et al.*, 2009; Ahmend and Shalaby, 2012; Ncube and Bvenura, 2012; Papenfus *et al.*, 2013; Xu and Leskovar, 2015). However, there is no information on the use of EM and seaweed concentrates combined in crop production. In some sustainable cropping systems where the two were applied separately, farmers have often cited application costs as the main challenge to adopt them as alternative fertilizers (Khaliq *et al.*, 2006; Pramanick *et al.*, 2014). Application costs of the two bio-stimulants consist of products price (EM = R100.00/litre and SWC Kelpak = R145.00/litre), labor and water needed. The prices are relatively high, especially for resource-poor farmers. However, the global market for bio-stimulants was projected to reach \$2,241million by 2018 and to have a compound annual growth rate of 12.5 % from 2013 to 2018 (Anonymous, 2013). In attempts to reduce the use of agrochemicals and produce crops in a sustainable manner, EM and SWCs have been combined with various organic and inorganic fertilizers to test their efficacy on various crops. Ncube *et al.*, (2011) combined EM with different soil amendments on tomato study to improve growth and yield. Khaliq *et al.*, (2006) reported that EM application increased the mineral nutrients (NPK) in cotton but was ineffective in increasing the yield alone. Cotton yield was however increased when the EM was combined with compost and NPK fertilizer (Khaliq *et al.*, 2006). Hu and Qi (2013) reported that long-term EM application in combination with compost enhanced straw biomass, grain yields, and grain nutrition of wheat. Several studies of SWC Kelpak® reported positive results on growth and yield of various agricultural crops. The seaweed concentrate increased the yield of greenhouse-grown tepary beans under different levels of nutrient supply (Beckett *et al.*, 1994). Papenfus *et al.*, (2013) found that the material enhanced a growth of okra (*Abelmoschus esculentus*) seedlings grown under deficient phosphorus and potassium with no effect under nitrogen deficient. Kelpak® significantly improved growth of tomato seedlings when applied as soil drench (Crouch and Van Staden, 1992). It was also found to have a positive effect when applied with NPK fertilizer on growth of the aboveground biomass of cocksfoot (*Dactylis glomerata*), with the use of 40% and 60% Kelpak® solution showing the highest efficiency (Sosnowski *et al.*, 2013). Application of

Kelpak® in a medium receiving half strength of chemicult (inorganic fertilizer) increased number of fruit and fruit size of three varieties of peppers (*Capsicum annum*) (Arthur *et al.*, 2003). Seaweed concentrates were also found to be effective under stress conditions (Papenfus *et al.*, 2013; Xu and Leskovar, 2015). However, the efficacy of the two bio-stimulants depends on crop species, climatic conditions, frequent and application method among others (Yamanda and Xu, 2000; Khalid *et al.*, 2004; Craigie, 2014; Calvo *et al.*, 2014). Other researchers have reported no effect when the two bio-stimulant were used singly (Javaid, 2009; Papenfus *et al.*, 2012) or in combination with other organic or inorganic fertilizers (Fraszczak *et al.*, 2012; Ncube and Bvenura, 2012; Lola-Luz *et al.*, 2014). Regardless of the pros and cons associated with these two bio-stimulants in crop production, their combined effect on growth and yield of any crop has never been documented. Thus, the aim of the study was to investigate combined effect EM and Kelpak® on growth and yield of cabbage using their recommended application rates.

Materials and Methods

Experimental Sites, Design and Treatments

Experiments were carried out in Plant Production Shade House (23°88' 06"S, 29°73' 39"E) and in the micro-plot at Experimental Farm, Syferkuil (23°53' 96"S, 29°43' 48"E) of the University of Limpopo. Under both sites, experiments were laid out in a randomised complete block design (RCBD) with four treatments, which were control (T₀), Kelpak® (T₁), EM (T₂), and EM+Kelpak® (T₃), with 10 replicates.

Experimental Procedures

Effective microorganisms stock solution and Kelpak® were obtained from Micro-zone Polokwane cc, Polokwane, South Africa, and Kelp Products Pty Ltd, Simons' Town, Cape Town, South Africa, respectively. In both shade house and micro-plot experiments, mixture of steam-pasteurised sand, Hygromix (Hygrotech, Tzaneen, South Africa) and Cultera Compost (Cultera [Pty] Ltd, Johannesburg, South Africa) at a ratio of 1:1:1 (v/v/v) was used as a growing medium. After filling 30 cm diameter plastic pots with growing medium, pots were covered with black plastic for one month to allow decomposition and nutrients mineralisation of the growing medium (Ncube *et al.*, 2011). Pots that were assigned to receive T₂ and T₃ were irrigated with 400 ml of EM diluted with water at a ratio of 1:100 (v/v) every other week prior to transplanting. Whilst pots assigned for T₀ and T₁ received 400 ml of water only. Water used in the study was de-chlorinated by allowing it to stand for 24 hours under room temperature to prevent killing of microorganisms in EM by chlorine (Zimmermann and Kamuenjandje, 2008).

Four-week-old seedlings of cabbage cv. Optima were purchased from M/D nursery (Tzaneen, South Africa).

Transplanting was done one month after pots were laid out in both shade house and micro-plot. Each treatment was diluted with water at a ratio of 1:100 (v/v) (Zimmermann and Kamuenjandje, 2008; Stirk *et al.*, 2014), whereas, control treatment was a pure, undiluted water. The application of treatments commenced immediately after seedlings transplanting and was repeated at 7-day interval until the termination of experiments. The treatments were applied as soil drench at an application rate of 400 ml per plant/pot. No additional irrigation was done except for application of treatments. Foliar insect pests like aphids (*Brevicoryne brassicae*) and diamondback moth (*Plutella xylostella*) were controlled twice using Chlorpyrifos EC and Karate Zeon EC after they were scouted. Fusarium wilt (*Fusarium oxysporum*) was controlled once using Bravo 720 after symptoms were noticed. Weeding was done manually when necessary. Application of all insecticides and fungicides used was according to manufactures' recommendations. Plant height was measured every second week using a ruler. It was measured from collar to highest leaf. Number of fully expanded leaves (leaves with clear visible petiole) was counted every 2nd week. Measurements were taken 2 weeks after transplanting up to 6 weeks. Harvesting was carried out 4 months after transplanting. From all experimental units, fresh weight of entire cabbage with wrapper leaves and roots was measured using a weighing balance (Adam® equipment-Model: AE75801138). Plant was separated into root, wrapper leaves and head after which their fresh weight was measured. Diameter of stem was measured using digital vernier caliper. Leaves and roots were oven dried at 65°C to constant dry weight for 4 days (96 hours), thereafter, their dry weight was measured Muthaura *et al.*, (2010). Head was cut longitudinally, after which head diameter was measured in two directions: polar and equatorial, using a ruler. Cut head was further cut into small pieces, then oven dried at 65°C to constant dry

weight for 6 days (144 hours) thereafter, their dry weight was measured.

Statistical Analysis

All data collected were subjected to analysis of variance (ANOVA) using Statistix 9.0 (Statistix Analytical Software, 1985 to 2009). Treatment means were separated by Least Significant Difference (LSD) at probability level of 0.05.

Results

Growth Variables

At week 2 and 4, treatments did not differ significantly ($P > 0.05$) under micro-plot (Table 1) and shade house (Table 2) on number of leaves counted. However, at week 6 treatments differed significantly ($P \leq 0.05$) under shade house but did not differ under micro-plot. At week 6, under shade house, EM and Kelpak® singly and in combination significantly increased number of leaves compared to the control. For seedling height, at week 2 and 4, treatments did not differ significantly ($P > 0.05$) under both sites (Table 1-2). However, at week 6 treatments differed significantly ($P \leq 0.05$) under micro-plot, highly significant ($P \leq 0.01$) under shade house. At week 6, under micro-plot, EM significantly decreased seedling height compared to other treatments. However, under shade house, EM and Kelpak® singly and in combination significantly increased number of leaves compared to the control. Control and EM produced the least seedling height under micro-plot and shade house, respectively. Treatments were significantly high ($P \leq 0.04$) under both sites on chlorophyll content (Table 3-4). Under both sites, treatment EM+Kelpak® produced significantly the highest chlorophyll content compared to Kelpak® and control. However, it did not differ significantly with EM. Application of EM resulted in increased chlorophyll content as compared to the control but not with Kelpak®.

Table 1: Treatments effect on growth variables of cabbage grown under micro-plot (n= 10).

Treatments	Number of leaves			Seedling height (mm)		
	Week 2	Week 4	Week 6	Week 2	Week 4	Week 6
Con	8.20 ^a	9.20 ^a	12.20 ^a	19.90 ^a	23.80 ^a	24.62 ^a
Kelp	7.90 ^a	8.90 ^a	12.60 ^a	19.05 ^a	22.50 ^a	24.20 ^a
EM	8.60 ^a	8.20 ^a	12.40 ^a	17.00 ^a	20.35 ^a	18.50 ^b
EM+Kel	9.00 ^a	8.90 ^a	14.10 ^a	20.45 ^a	22.44 ^a	25.00 ^a
Significant	ns	ns	ns	ns	ns	*
LSD-value	-	-	-	-	-	4.95

Means followed by the same letter in a column were not significantly different at $P > 0.05$, * = $P \leq 0.05$, ** = $P \leq 0.01$, ns = non-significant at $P > 0.05$.

Table 2: Treatments effect on growth variables of cabbage grown under Shade house (n= 10).

Treatments	Number of leaves			Seedling height (mm)		
	Week 2	Week 4	Week 6	Week 2	Week 4	Week 6
Con	8.40 ^a	8.40 ^a	5.80 ^b	14.45 ^a	18.59 ^a	15.80 ^b
Kelp	8.30 ^a	9.30 ^a	7.80 ^a	15.50 ^a	19.58 ^a	21.05 ^a
EM	8.20 ^a	9.50 ^a	7.90 ^a	15.08 ^a	20.98 ^a	22.20 ^a

EM+Kel	8.20 ^a	9.40 ^a	8.00 ^a	15.56 ^a	20.18 ^a	21.20 ^a
Significant	ns	ns	*	ns	ns	**
LSD-value	-	-	1.77	-	-	4.08

Means followed by the same letter in a column were not significantly different at $P > 0.05$, * = $P \leq 0.05$, ** = $P \leq 0.01$, ns = non-significant at $P > 0.05$.

Yield Variables

Treatments differed significantly ($P \leq 0.05$) under both sites on entire weight (Table 3-4). Treatment EM+Kelpak® and EM increased entire cabbage weight as compared to Kelpak®. However, control neither decreased nor increased the variable compared to the other three treatments. Kelpak® produced cabbage with the least entire weight among the treatments. Treatments also differed significantly ($P \leq 0.05$) under both sites on fresh leaf weight (Table 3-4).

Table 3: Treatments effect on yield variables of cabbage grown under micro-plot (n= 10).

Treatment	Chloro- phyll content (%)	Entire plant weight (kg)	Fresh leaf weight (kg)	Fresh head weight (kg)	Fresh root Weight (g)	Dry leaf weight (g)	Dry head weight (g)	Dry root weight (g)	Head equatorial diameter (cm)	Head polar diameter (cm)	Stem Diameter (mm)
Con	51.67 ^c	1.85 ^{ab}	0.58 ^b	0.94 ^{bc}	51.94 ^a	72.62 ^a	126.72 ^a	17.04 ^a	10.35 ^a	9.80 ^a	31.31 ^b
Kel	63.70 ^{bc}	1.25 ^b	0.47 ^b	0.81 ^c	37.01 ^a	79.51 ^a	107.60 ^a	12.85 ^a	12.35 ^a	12.15 ^a	37.91 ^{ab}
EM	78.87 ^{ab}	2.53 ^a	0.67 ^{ab}	1.68 ^{ab}	49.62 ^a	96.19 ^a	215.54 ^a	15.79 ^a	14.58 ^a	14.05 ^a	42.63 ^a
EM+Kel	90.19 ^a	2.70 ^a	0.92 ^a	1.95 ^a	51.41 ^a	127.82 ^a	263.59 ^a	15.68 ^a	14.95 ^a	15.35 ^a	44.18 ^a
Significant	**	*	*	*	ns	ns	ns	ns	ns	ns	*
LSD-value	20.97	1.10	0.32	0.85	-	-	-	-	-	-	9.26

Means followed by the same letter in a column were not significantly different at $P > 0.05$, * = $P \leq 0.05$, ** = $P \leq 0.01$, ns = non-significant at $P > 0.05$.

Table 4: Treatments effect on yield variables of cabbage grown under Shade house (n= 10).

Treatment	Chloro- phyll content (%)	Entire plant weight (kg)	Fresh leaf weight (kg)	Fresh head weight (kg)	Fresh root weight (g)	Dry leaf weight (g)	Dry head weight (g)	Dry root weight (g)	Head equatorial diameter (cm)	Head polar diameter (cm)	Stem Diameter (mm)
Con	41.66 ^c	1.82 ^{ab}	0.55 ^b	0.92 ^{bc}	34.74 ^a	63.61 ^a	111.96 ^a	8.95 ^a	08.85 ^a	08.30 ^b	23.31 ^b
Kel	53.70 ^{bc}	1.21 ^b	0.44 ^b	0.80 ^c	23.01 ^a	70.01 ^a	096.60 ^a	7.98 ^a	10.55 ^a	10.35 ^{ab}	31.89 ^a
EM	68.85 ^{ab}	2.52 ^a	0.64 ^{ab}	1.66 ^{ab}	31.25 ^a	85.10 ^a	199.69 ^a	9.69 ^a	12.88 ^a	12.65 ^a	32.65 ^a
EM+Kel	80.19 ^a	2.65 ^a	0.88 ^a	1.89 ^a	34.41 ^a	118.88 ^a	246.52 ^a	12.15 ^a	13.35 ^a	13.75 ^a	34.17 ^a
Significant	**	*	*	*	ns	ns	ns	ns	ns	*	*
LSD-value	20.97	1.10	0.32	0.84	-	-	-	-	-	4.01	7.24

Means followed by the same letter in a column were not significantly different at $P > 0.05$, * = $P \leq 0.05$, ** = $P \leq 0.01$, ns = non-significant at $P > 0.05$.

Treatment EM+Kelpak® increased fresh leaf weight compared to control and Kelpak®. However, EM neither decreased nor increased fresh leaf weight compared to other three treatments. Fresh head weight was found to be significantly ($P \leq 0.05$) affected by the treatments under both sites (Table 3-4). Under both sites, combination of EM and Kelpak® significantly increased fresh head weight compared to Kelpak® and control. However, EM when applied alone neither decreased nor increased fresh head weight when compared to EM+Kelpak and control, but significantly ($P \leq 0.05$) increased the variable compared to Kelpak®. Kelpak® produced plants of the least fresh head weight. Treatments did not differ significantly ($P > 0.05$) under both sites on fresh root, dry root, dry leaf and dry head weight (Table 3-4).

Treatments were significantly different ($P \leq 0.05$) under both sites on stem diameter. Under micro-plot (Table 3), treatment EM+Kelpak® and EM significantly increased stem diameter compared to the control. However, Kelpak® neither decreased nor increased stem diameter compared to control and other two treatments. Under shade house (Table 4), EM and Kelpak® singly and in combination significantly increased stem diameter compared to the control. Treatments were also differed significantly ($P \leq 0.05$) on head polar diameter under shade house (Table 4). Treatment EM+Kelpak® and EM significantly increased polar stem diameter compared to the control. However, Kelpak® neither decreased nor increased polar head diameter compared to control and other two treatments. Under micro-plot (Table 3), treatments were not significant ($P > 0.05$) on polar head diameter. Equatorial polar diameter was not significant ($P > 0.05$) under both sites (Table 3-4).

Discussion

Our data revealed that combination of EM and Kelpak® had effect on growth and yield of cabbage, although some of the measured variables were not statistically significant. Number of leaves was not significantly affected during the first four weeks under both micro-plot and shade house conditions. The observed seedling response was attributed to growing mixture being able to provide sufficient nutrients at early stage which improved growth of all cabbage plants irrespective of treatment applied. In agreement with our findings, Olle and Williams (2015) found that two weeks after transplanting pumpkin (*Cucurbita maxima*) in a beat-based mixture added with synthetic fertilizers, application of EM did not have effect on seedling growth of pumpkin. Also, Papenfus *et al.*, (2013) found that Kelpak® at week 8 had no effect on seedlings growth of okra (*Abelmoschus esculentus*) when seedlings were grown under enough nutrient condition.

At week 6 under shade house, there were significance differences among the treatments. Application of bio-

stimulants alone and in combination increased number of leaves compared to the control. This was in agreement with results of Abdel-Mawgoud *et al.*, (2010) and Muthaura *et al.* (2010), which indicated that SWC and EM increased watermelon (*Citrullus lanatus*) and pigweed (*Amaranthus dubians*) leaves number, respectively. This response was attributed to the presence of phytohormones from SWC which trigger physiological processes in plants. Decomposition and mineralisation by microorganisms in EM supply more nutrients into the rhizosphere which leads to plant growth and development (Krishnapriya *et al.*, 2012; Calvo *et al.*, 2014), hence observed increment in number of leaves on these treatments. Contrary to shade house, under micro-plot, treatments were not significantly different for cabbage leaves number at week 6. The results were in accordance with Idris *et al.*, (2008) and Craigie (2014), who reported that crops' response to EM and SWC was dependent on the environmental conditions. Seaweed concentrates were reported to be effective under stress conditions, with least response under optimal conditions (Craigie, 2014; Xu and Leskovar, 2015). How environmental conditions affect crops' response to EM is not clear. However, our results from both sites showed that environmental variations had influence on how plants responded to EM. This observation was supported by Idris *et al.*, (2008), who found that spraying tomato crops with EM under different growing conditions responded differently to a treatment.

During the first four weeks under both sites, results showed that treatments did not have effect on seedling height. This was consistent with the number of leaves at this stage. Therefore, this suggests that during early stages, cabbage produce leaves of approximately the same height regardless of treatment applied. However, at week 6, under both sites, treatments had significant effect on seedling height. Bio-stimulants alone or in combination produced seedlings of the same height compared to control under shade house. While under micro-plot, EM had negative effect compared to other treatments. The increase in seedling height under shade house could be attributed to cytokinins which triggered shoot formation and leaf development (Szabo and Hrotko, 2009). Decrease due to EM application could be attributed to nutrients immobilisation (Mayer *et al.*, 2010), which probably inactivated the release of beneficial bioactive substances from microorganisms in EM. Application of EM into the rhizosphere has been shown to result in increased soil microorganism populations, leading to rapid mineralisation of organic materials and the release of bioactive substances which are important for plant growth and development (Javaid and Bajwa, 2014). Application of EM and Kelpak® singly and in combination significantly increased chlorophyll content with combination of the EM and Kelpak® having the highest chlorophyll content under both sites. Khalid and Joo (2011) found that EM increased leaf chlorophyll of Chinese cabbage (*Brassica rapa*). The

presence of cytokines (Stirk *et al.*, 2004) and polyamines (Papenfus *et al.*, 2012) in Kelpak® and their release by microorganisms (Dong *et al.*, 2008) might have been responsible for increased chlorophyll content due to their effect in inhibiting degradation of chlorophyll and delaying leaf senescence (Elumalai and Rengasamy, 2012).

Results showed that treatments significantly influenced whole plant weight, fresh leaf and head weight and stem diameter of cabbage under micro-plot and shade house. Under shade house, head polar diameter was also significantly influenced by treatments. Combination of EM and Kelpak® increased these variables compared to either bio-fertilizer alone or the control, under both sites. Combination of bio-stimulants also increased dry head and leaf weight. In the present study under shade house, lack of pronounced effect on root growth could be attributed to surface hardness of growing medium observed. However, this was not seen under for micro-plot.

Lola-Luz *et al.*, (2013) and Lola-Luz *et al.*, (2014) found that SWC prepared from *Ascophyllum nodosum* did not increase the yield of cabbage and broccoli (*Brassica oleracea*), respectively. Similarly, Ncube *et al.*, (2014) found that EM did not increase the yield of tomato. However, combination of SWC and compost increased yield of cucumber (Ahmend and Shalaby, 2012). Also, EM was found to be more effective in increasing the yield of wheat when combined with compost (Hu and Qi, 2013). In the present study, combination of EM and Kelpak® was found to be more effective in increasing yield of cabbage. Increase in yield was attributed to EM acting as a source of nutrients through mineralisation, while Kelpak® helping in uptake of the available nutrients. Seaweed concentrates enhance plant growth and yield by improving nutrient uptake by roots (Zodape *et al.*, 2014). Also, the presence of macro and micronutrients and phytohormones in Kelpak® might have played a role in increasing the yield (Sharma *et al.*, 2014; Stirk *et al.*, 2014). Seaweed concentrates have been reported to increase the population and activity of soil microorganisms (Alam *et al.*, 2013). Therefore, combination of EM and Kelpak® might have increased nutrients mineralisation compared to where EM was applied alone. The results showed that combination of EM and Kelpak® increased dry matter accumulation and stem diameter. These results might support the idea that EM could have increased available nutrients and Kelpak® facilitated their uptake by cabbage plants. Increase in stem diameter was found to help plants obtain better nutrients from the soil (Olle and William, 2015).

Results showed that treatments did not influence fresh and dry root weight, equatorial diameter, and head shape of cabbage under micro-plot and shade house. Head polar diameter was influenced by treatments under micro-plot with the exception under shade house. To support our findings, Khalid and Joo (2011) found that EM did not significantly increase root fresh weight of Chinese cabbage. Also, Papenfus *et al.*, (2013) found that Kelpak®

failed to increase root growth of okra under nutrients stress-free condition but increased the variable under potassium and phosphorus stress. Contrary to our findings, Muthaura *et al.*, (2010) found that EM increased fresh and dry weight of roots of pigweed. Seaweed concentrate prepared from *Hypnea musciformis* was also found to have increased fresh and dry root weight of groundnut (*Arachis hypogea*) (Selvam and Sivakumar, 2014). With regard to diameter, Fawzy, *et al.*, (2012) found that foliar application of EM positively influenced bulb diameter of onion. This was also shown by Sutharsan *et al.*, (2014) that foliar application of SWC of *Sargassum crassifolium* increased polar diameter of tomato with no effect to its equatorial diameter. Increase of root weight and length was attributed to auxins in SWC (Craigie, 2014; Calvo *et al.*, 2014), while EM increases root growth through secretion of useful bioactive substances such as amino acids, hormones and enzymes (Hussain *et al.*, 2002).

Conclusion

Combination of EM and Kelpak® significantly increased number of leaves, chlorophyll content and seedling height of cabbage. The study further found that yield was increased due to combined application of EM and Kelpak®, although most of the variables were not statistically significant. Further research is needed to test this combination of bio-stimulants under field conditions. Again, how combination of bio-stimulants increased growth and yield of cabbage (mechanism) was not studied; therefore, work is needed to evaluate the mechanisms involved.

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