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Influence of Organic Manure and Inorganic Fertilizer on the Growth, Yield and Phytochemical Constituents of Cabbage (*Brassica oleracea*)

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Authors' contributions

This work was carried out in collaboration among all authors. Author SMO designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors III and ZAR managed the analyses of the study. Author ZAR managed the literature searches and carried out the field work. All authors read and approved the final manuscript.

Article Information

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Original Research Article

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ABSTRACT

Organic farming is gaining attention and increasing globally because of its eco-friendly, safety and its health benefits to humans. A field experiment was conducted at Federal College of Forestry Jos, to determine the influence of organic manure and inorganic fertilizer on the growth, yield and phytochemical constituents of cabbage. Randomized Complete Block Design was used as experimental design involving five treatments with T_0 as control (No application of manure), T_1 (N.P.K fertilizer), T_2 (Poultry droppings), T_3 (Cow droppings) and T_4 (Goat droppings). Data was taken on plant height, number of leaves, head diameter and head weight of cabbage. Qualitative phytochemical analysis on saponins, tannins, alkaloids, flavonoids, cardiac glycosides, anthocyanins, phenols, amino acids, steroids and terpenoids were carried out and quantitative analysis was done for phytochemicals present. The result obtained showed a significant difference for the plant height, leaf count, head diameter and head weight at $p \le 0.05$. Flavonoids, alkaloids, amino acids, terpenoids, tannins and phenols were present in the qualitative analysis and at different rates. Cabbage cultivation with poultry droppings (T_2) was observed to stand out from the

other treatments for the yield parameters and the phytochemical analysis. Result of the quantitative phytochemicals revealed that more phenolics, alkaloids and flavonoids were present in cabbage grown with organic manure than in inorganic fertilizer.

Keywords: Cabbage; organic manure; inorganic fertilizer; phytochemicals; growth; yield.

1. INTRODUCTION

The major essence of crop cultivation is for consumption in order to derive nutrients for proper growth and development of the body. Even though nutrients are derived from food crops, is it undisputable that food crops performs medicinal functions to the body. This is more so when organically grown food crops are consumed as compared to those grown with inorganic fertilizer. Hence, the advocate for the consumption of organically grown food crops is increasing on a daily basis. Vegetable are mostly encouraged to be grown with the use of organic manures in order for them to deliver both nutritional functions and medicinal functions. Cabbage as a popularly consumed vegetable is not left out in this discussion.

Cabbage, Brassica oleracea is from the plants known as cole crop. Cabbage was domesticated about 2000 years ago [1]. It is a multi-layered vegetable which is cultivated majorly in India, China, and North America and in Africa it is majorly grown in Malaysia, Tanzania and in Nigeria. Cabbage is commonly cultivated in Nigeria and widely consumed raw and cooked by humans for vitamin and mineral nutrients [2]. In 2014, global production of cabbages was estimated at 21.8 million tonnes, with China being the highest producer with 47% production of the World total production. Other major producers are India with 9.0 million metric tonnes, Russia 3.5 million metric tonnes [3]. In Nigeria cabbage is mostly cultivated in the Northern parts. In 2016, cabbage production grew from 2.7 million to 14.3 million tonnes.

For healthy growth and yield of cabbage, nutrients must be applied and it should be in the correct proportion [4]. Crop cultivation with the use of different manures gives differences in nutritional output of crops. This is because the nutrients released by these manure from animal waste depend largely on the type of feeds and the nutrients contained in such feed [5]. Research studies [6,7] shows that availability of plant nutrients can influence the availability of phytochemical constituents/secondary metabolites in plants. In comparism with the

organic manure, the use of chemical fertilizer provides nutrients within a short period of time and on the long run have residual effect in the soil [8]. Also, it is widely believed that the use of chemical fertilizer could cause water and soil pollution [9]. Fertilizer usage can also affect the rate of synthesis of phytochemicals in plants and thus reduce their medicinal content [10]. Nutrients in the form of organic manure and inorganic fertilizer has been reported to have an influence on the phytochemical and nutritional quality of crops [11]. Cabbage is a rich source of Vitamin C and vitamin K, containing 44% and 72% respectively per 100 gram [12]. Basic research on cabbage phytochemicals is ongoing to discern if certain cabbage may affect health or have anti diseases effects. Such compounds include sulforaphane and other glucosinolates which may stimulate the production of detoxifying enzymes during metabolism [13]. Cabbage is good for the eyes and skin; they lower cholesterol level in the body and have antioxidant that prevents cancer [14]. Isothiocyanate, an anti-cancer compound extracted from cabbage was discovered through phytochemical profiling of cabbage.

Phytochemicals also known as phytonutrient or secondary metabolites which are non-nutritive plant chemicals synthesized by plants for their protection against predators and herbivore. These complex molecules are found in most foods, especially in fruits and vegetables. It was assumed that vitamins and minerals play a significant role in disease prevention however, recent studies indicates that phytochemicals can make a much greater contribution than vitamins nutrients. Phytochemicals or other are associated with the prevention of certain chronic diseases, including cardiovascular diseases, cancer, diabetes, osteoporosis and vision diseases [15]. Phytochemicals in fruits and vegetables like cabbage are mostly responsible for a protective effect against these diseases. Chronic diseases such as cancer are been prevented through the use of phytochemicals and their cure also depends largely on phytochemical usage. Also, phytochemicals can reduce the rate of cancer spread by slowing down the proliferation of cancer cell [16].

Phytochemical compounds have the ability to ameliorate various diseases by either preventing such diseases or curing them. Tannin is reported to have antimicrobial activity and antibacterial activity. Alkaloids have also been reported to act as antioxidant which engulfs reactive oxygen species in the body system [17].

2. MATERIALS AND METHODS

The experiment was conducted at Federal College of Forestry, in Jos North Local Government Area of Plateau State. Jos is located on a plateau with an elevation of about 1200 m above the sea level. It is between 7-11° North latitude and 7-8° East longitude. Jos is located in the middle belt of Nigeria with mixed weather of temperate and tropical climate and relative humidity of 60%. The soil is sandy loam and light to dark brown in colour. Temperature range is between 10-32°C and the mean annual rainfall is about 1340 mm [18].

2.1 Sources of Materials

The materials used for planting include three sources of organic manure which are poultry droppings, cow dung and goat droppings which are gotten from various animal farms. Nitrogen Phosphorus and Potassium (NPK) fertilizer, cabbage seeds were bought from Plateau Agricultural Development Program (PADP), Dogon Dutse in Jos North local government area of Plateau State. Cutlass, hoe, measuring tape, pegs, watering can, weighing balance knife were the planting materials used and also for harvesting. Some of the laboratory reagents and equipments are rotary evaporator, distillation flask, water bath, spectrophotometer, electric blender, ferric chloride, sodium hydroxide, chloroform, hydrochloric acid, ninhydrin, ethanol, soxhlet extractor, 10% ammonia solution, tetraoxosulphate (vi) acid, sulphuric acid and acetic acid. The reagents were gotten from Sigma Aldrich, all collected from Biochemistry Laboratory and were of analytical grade.

2.2 Methods

A nursery bed of 2 square meters was constructed and the soil was loosened. Cabbage seeds were properly mixed with sharp sand and then broadcasted on the nursery bed. The seedlings germinated on the second day and they were allowed to reach a height of 12 cm at 5 weeks after planting before transplanting according to the method of [19]. A total plot size of 10.5 m X 13 m was constructed with 20 beds of 2 m X 2 m, after which the various manure was applied 3 weeks before transplanting of the cabbage seedlings for proper decomposition after which the soil was tilled. An inter-row spacing of 40cm and intra-row spacing of 40 cm was adopted. After 3 weeks of transplanting 40g of N.P.K fertilizer was applied to T₁ and weeding was carried out properly to prevent crop weed competition. Harvesting was done at maturity stage by using hand and knife. The cabbage leaves were peeled and properly washed with clean water after which it was dried at room temperature in the laboratory. It was then grinded and pulverized to finely divided powder for phytochemical analysis.

2.3 Phytochemical Analysis

The yield obtained was macerated and air dried until total dry mass was obtained. The dried sample was pulverized using an electric blender after which it was weighed and then percolated with equal volume of ethanol for 24 hours. The resulting solution was then filtered and the filtrate obtained containing the bioactive compounds was placed in a rotary evaporator to obtain the crude extract and recover the solvent. The method described by Makkar et al. [20] was used to determine the presence of saponin, flavonoid, Alkaloid. Terpenoids. Tannins. cardiac glycosides present in the ethanolic extract using first principle at the laboratory of Federal College of Forestry. Jos. The quantitative phytochemical analysis was conducted based on method described by Ekwueme et al. [21] and Ibrahim et al. [22].

2.3.1 Procedure of phytochemical analysis

- i. Flavonoids: Sodium hydroxide (1 ml of 2 n) was added to 1 ml of each plants extract, yellow color indicates presence of flavonoids.
- ii. Tannins: ferric chloride (2 m of 5%) was added to 1 ml of each the plant extracts and the formation of dark blue or greenish black indicates the presence of tannins.
- iii. Saponin: Distilled water (2 ml) was added to 2 ml of each extracts and shaken in graduated cylinder of 15 minutes length wise. The formation of 1 cm layer of foam indicates the presence of saponin.
- iv. Cardiac glycoside: Chloroform (3 ml) and ammonia solution (10%) was added to 2 ml of plant extract. Formation of pink color indicates the presence of glycosides.

- Alkaloid; concentrated hydrochloric acid (2 ml) was added to 2 ml of each extract, then few drops of mayers reagent was added. Presence of green color or white precipitate indicates the presence of alkaloids.
- vi. Amino acid: Ninhydrin test was used for amino acid analysis. Few drops of 0.2% ninhydrin was added to 2 ml of plant extract and heated for 5 minutes formation of blue color was indicated.
- vii. Terpenoids: 5 ml of each extract was mixed in 2 ml of chloroform and concentrated sulphuric acid (3 ml) was carefully added to form a layer. A reddish brown precipitation of the interface indicates the presence of terpenoids.
- viii. Anthocyanin: Sodium hydroxide (1 ml of 2N) was added to 2 ml each of plants extract and heated for 5 minutes at 100%. Formation of bluish green color indicates the presence of anthocyanin.
- ix. Phenol: Distilled water (2 ml) followed by few drops of 10% ferric chloride was added to 1 ml of the extracts. Formation of blue or green colour indicates the presence of phenols.
- x. Steroids: The extracts were dissolved in 2 ml of chloroform to which 10 drops of acetic acid and 5 drops of concentrated tetraoxosulphate (vi) acid was added and mixed. The change of red color through blue to green indicates the presence of steroids.

2.4 Experimental Design

The experiment was made up of Randomized Complete Block Design (RCBD) as the experimental Design. Five treatments were used and the treatments consist of T_0 , T_1 , T_2 , T_3 and T_4 which were all replicated four times.

- i. T₀ = Control experiment in which no manure or fertilizer was applied
- ii. T_1 = consisted of 40 g of NPK 15:15:15 fertilizer applied per 4 m² plot
- iii. T_2 = Cow dung manure was applied at the rate of 8 kg per 4m² plot
- iv. T_3 = Poultry droppings manure was applied at the rate of 8 kg per 4 m² plot
- v. T_4 = Goat dropping manure was applied at the rate of 8 kg per 4 m²

2.5 Growth Parameters Collected

i. Weight of Head: Three head samples were randomly selected from each plot and

their weight was taken immediately after harvesting. The mean value was used for the statistical analysis.

- ii. **Diameter of Head:** The diameter of the randomly selected heads were measured using a vernier caliper. The mean of the three samples was used for further analysis.
- Leaf Count: The numbers of leaves were counted for the selected sample plants at 2 weeks after transplanting (2WAT), 4 WAT, 6 WAT, 8 WAT and 10 WAT and the average value was recorded.
- iv. Plant Height: The plant height was measured with a meter rule and the average value was recorded. The mean value was subjected to statistical analysis.

2.6 Statistical Analysis

All data collected was subjected to one way analysis of variance (ANOVA) at 5% level of significance using statistical package for social students (SPSS) Version 23. Duncan Multiple Range Test (DMRT) was used to separate the means where differences occur in the means.

3. RESULTS AND DISCUSSION

3.1 Plant Height

Table (1) shows the effect of different manure and inorganic fertilizer on the height of cabbage. The result shows that at 2 WAP and 4 WAP, there is no significant difference on the height of the cabbage. At 6WAP, a significant difference occurs with the highest mean value observed in T_2 (18.03). T_4 with mean value of 17.70 is however not significantly different from T_2 and T_3 is seen to be statistically similar to T₂ and T₄. At 8WAp, significant difference occurred with the h9qhest mean value observed in T₄ (19.95). This is similar to the result obtained at 10WAP in which the highest mean value occurred in T₄ (20.90) and it is significantly different from the control (15.50), T_1 (18.10), T_2 (19.43) and T_3 (19.00). The work of [23] on Amaranth as well as that of [5] showed that poultry manual and other organic means of adding nutrients to plants gave significant effect in term of plant height compared to the control and this clearly revealed similarities with this research work as observed in Table (1). This could also be that earlier application of organic manure before planting brought about proper decomposition and immediate release of nutrient which caused rapid growth performance and led to increase in plant height [24].

Treatments	2WAT	4WAT	6WAT	8WAT	10WAT
T ₀	2.88 ^a	11.40 ^a	13.65 ^a	14.68 ^a	15.50 ^a
T ₁	2.33 ^a	12.73 ^a	13.58 ^ª	16.80 ^{ab}	18.10 ^{ab}
T_2	3.08 ^a	12.80 ^a	18.03 ^b	19.25 ^b	19.43 ^b
T_3	2.68 ^a	13.43 ^a	15.10 ^{ab}	18.30 ^b	19.00 ^b
T ₄	3.05 ^ª	10.73 ^a	17.70 ^b	19.95 ^b	20.90 ^b
S.E±	0.4704	2.249	1.45425	1.43721	1.46796
LS	N.S	N.S	*	*	*

 Table 1. Effect of different organic manure and inorganic fertilizer on cabbage height at 2WAP, 4WAP, 6WAP, 8WAP and 10WAP

Means within a column having same letters are not significantly different at $P \le 0.05$; LS = level of significance; * = Significant at 0.05, N.S = Not Significant; S.E = Standard Error

Table 2. Effect of different organic manure and inorganic fertilizer on the number of leaves of cabbage at 2WAP, 4WAP, 6WAP, 8WAP and 10WAP

Treatments	2WAT	4WAT	6WAT	8WAT	10WAT
To	3.80 ^a	8.38 ^a	10.18 ^a	11.18 ^a	11.95 ^a
T ₁	4.88 ^{ab}	10.05 ^b	12.48 ^b	13.73 ^b	14.85 ^b
T_2	5.05 ^{ab}	11.08 ^b	12.13 ^b	13.63 ^b	15.23 ^b
T ₃	5.88 ^b	11.50 ^b	11.83 ^b	13.80 ^b	14.65 ^b
T ₄	4.58 ^a	10.80 ^b	11.70 ^b	13.55 ^b	15.38 ^b
S.E±	0.56487	0.78028	0.65038	0.80657	1.15072
IS	*	*	*	*	*

Means within a column having same letters are not significantly different at $P \le 0.05$; LS = level of significance; * = Significant at 0.05; S.E = Standard Error

3.2 Leaf Count

The result of the leaf count shows significant difference at 2WAP, 4WAP, 6WAP, 8WAP and 10WAP. At 2WAP, T₃ (5.88) is observed to be significantly different from T_0 (3.80) and T_4 (4.58), and it is statistically similar to the results obtained in T₁ and T₂ (5.05). At 4WAP, T₃ has the highest mean value and it is statistically similar to T_1 , T_2 and T₄. It is however significantly different from the control experimental treatment (T_0) as shown in Table 2. The result obtained at 6WAP is similar to that of the result at 4WAP only that, the highest mean value at 4WAP occurred in T₁ (the plot with the application of NPK fertilizer), which has mean value of 12.48. Treatments T₁, T₂, T₃ and T₄ are statistically similar to one another and they are all significantly different from T₀. The existence of the significance difference between the treatments and the control could be attributed to the little nutrients available to the crop in the control compared with the experimental treatments. Organic manure are known to provide nutrients to the soil in the form of organic matter. Mineralization of this organic matter to provide the different nutrients required by the plant could be slow but highly effective, which support plant growth [25]. The application of organic manure helps plants to provide nutrients for the formation of leaves [26]. The nutrient supply from organic manure has been discussed to support plant root development rapidly and this could enhance the growth of the leaves [27].

3.3 Head Diameter

The result of the head diameter shows a significance difference across all the treatments. In comparism with the control with a mean value of 6.55, T_1 (7.01), T_2 (7.42), T_3 (7.79) and T_4 (8.15) are significantly different from T_0 (19.35). This indicates the effect of the various manure and fertilizer applied to the cabbage which support the growth and therefore translated to bigger head diameter. T_1 and T_2 are however statistically similar, likewise T₂, T₃ and T₄ when compared with one another as shown in (Table 3). Treatment T_4 possess the highest mean value of 8.15. This differential effect could be due to the release of various macronutrients and micronutrients which are required by cabbage in the enlargement of its size, and hence increase in the head diameter as observed in the various experimental treatments when compared with the control. This can be compared with the result obtained by [28] in which cultivation of Chinese cabbage under the influence of organic manure produced the highest mean value when

Treatments	Mean head diameter (cm)	
T ₀	6.55 ^a	
T ₁	7.01 ^{ab}	
T ₂	7.42 ^{abc}	
	7.79 ^{bc}	
T₄	8.15 ^c	
s.E±	0.42	
LS	*	

Table 3. Effect of different organic manure and inorganic fertilizer on the head diameter of cabbage

Means within a column having same superscripts are not significantly different(P ≤ 0.05); LS = level of significance; * = Significant at 0.05; S.E = Standard Error

compared with the control. The introduction of organic manure to soils enhance the microbial activity and thereby increase the fertility of the soil and productivity by loosening the soil particles and minerals [29]. Also, organic manure has been proved to significantly promote plant growth, head yield of broccoli [30].

3.4 Weight of Cabbage Head

The analysis result as shown in Table (4) revealed that significant difference occurs between the different treatments and the control on the weight of cabbage head. The lowest mean value is observed to be in T_0 (19.35), while T_4 has the highest mean value (32.75). Treatments T_4 (32.75) and T_2 (32.55) are also seen not to be significantly different from each other which makes them to be at par in term of yield. Soil nutrients released from the manure and inorganic fertilizer are absorbed by the plants through the cationic exchange of the proton (H^{\dagger}) in the root hair of plants and the nutrients in the soil. The absorbed nutrients, together with sunlight and water are used by plants in growth formation and increase in yield which could be correlated to increase in the weight of head in cabbage. The significant difference observed is in tandem with the published work of [5] in which poultry manure and goat droppings gave the highest mean value in tomato.

3.5 Qualitative Analysis

The phytochemical analysis revealed the possible medicinal properties of cabbage, making use of ethanol as solvent for extraction. The result shows that all the treatments contained flavonoid, tannins, amino acid, terpenoids and phenols. Tannins and steroid were both found only in T_2 . Treatments T_0 , T_1 and T_3 also contained tannins but steroid was also found to exist in T_4 apart from T_2 . The result

obtained shows that more phytochemicals are found in cabbage cultivated with poultry manure than those cultivation with inorganic manure, cow dung and goat droppings. This findings comply with the result of [17] in which their phytochemical profiling of cabbage indicated the presence of alkaloids, flavonoids, tannins, phenols and amino acid which correlates with the result in Table (5). Their result also showed the absence of steroids and saponin just as it was obtained in this research work in Table (5). Anthocyanin and cardiac glycoside were also absent in the ethanolic extract of the cabbage cultivated with different manure and inorganic acid [31]. Previous works on cabbage [32] reported the antibacterial, antifungal properties and anti-cancer properties. These various medicinal properties can be attributed to the different phytochemicals present in the extract. Tannin has been reported to have antibacterial activity as well as microbial activities [17]. Alkaloids are reported to possess antioxidant properties.

3.6 Quantitative Analysis

The result of the quantitative phytochemical analysis shows that the use of manure and fertilizer in cabbage cultivation could bring about significance increase in cabbage а phytochemicals. Flavonoids, alkaloids, amino acid and phenols showed distinct differences in the quantitative result for the different treatments when compared with the control. Poultry droppings (T₂)was observed to have the highest mean value in terpenoids (0.6300), flavonoids (0.4300), tannins (0.4200), alkaloids (0.5400), amino acids (0.5300) and phenols (0.4300) when compared with the other treatments. T₂, T₃ and T₄ were found to be statistically similar in term of the quantity of flavonoid contained in them. T₀, T₂ and T₃ produced the highest mean values for the quantitative tannins and T_2 , T_3 and T_4 are also

Treatments	Mean weight of head	
To	19.35 ^a	
T ₁	21.15 ^b	
T ₂	32.55 ^d	
$\overline{T_3}$	30.75 ^c	
T₄	32.75 ^d	
S.E±	0.36	
IS	*	

Table 4. Effect of different organic manure and inorganic fertilizer on the mean head weight ofcabbage

Means within a column with similar superscripts are not significantly different ($P \le 0.05$); LS = level of significance; * = Significant at 0.05; S.E = Standard Error

Table 5. Qualitative phytochemical analysis of cabbage grown with different organic manure and inorganic fertilizer

S/N	Phytochemicals	T ₀	T ₁	T ₂	T ₃	T ₄
1.	Flavonoids	+	+	+	+	+
2.	Tannins	+	+	+	+	-
3.	Saponin	-	-	-	-	-
4.	Cardiac Glycosides	-	-	-	-	-
5.	Alkaloids	+	+	+	+	+
6.	Amino Acids	+	+	+	+	+
7.	Terpenoids	+	+	+	+	+
8.	Anthocyanins	-	-	-	-	-
9.	Phenols	+	+	+	+	+
10.	Steroids	-	-	+	-	+

+ = Presence of Phytochemical in the cabbage extract; -= Absence of phytochemical in the cabbage extract

Table 6. Quantitative phytochemical analysis of cabbage grown with different organic manure and inorganic fertilizer

Treatments	Flavonoids (mg/g)	Tannins (mg/g)	Alkaloids (mg/g)	Amino acids (mg/g)	Terpenoids (mg/g)	Phenols (mg/g)
T ₀	0.2200 ^a	0.4200 ^b	0.2600 ^a	0.2700 ^a	0.0800 ^a	0.0500 ^a
T ₁	0.3400 ^b	0.3400 ^a	0.4300 ^c	0.4300 ^b	0.4800 ^b	0.3367 ^b
T_2	0.4300 ^c	0.4200 ^b	0.5400 ^d	0.5300 ^c	0.6300 ^c	0.4300 ^c
T ₃	0.4300 ^c	0.4200 ^b	0.2800 ^a	0.2600 ^a	0.4300 ^b	0.4300 ^c
T ₄	0.4300 ^c	0.3300 ^a	0.3400 ^b	0.3000 ^a	0.4400 ^b	0.4133 ^c
S.E±	0.01592	0.01506	0.01592	0.02000	0.01789	0.01402
LS	*	*	*	*	*	*

Means within a column having same superscripts are not significantly different (P ≤0.05); LS = level of significance; * = Significant at 0.05; S.E = Standard Error

statistically similar for the result of phenol contained in cabbage. [4] reported that total phenolics and flavonoid content were influenced by different manure and fertilizer rates, and also observed that application of organic manure brought about an increase in the production of flavonoids as well as phenolic content in the plant studied *Labisia pumila* Benth. The use of inorganic fertilizer at the rate of 40g/4m² and 8kg of goat droppings did not favor the result of tannins quantitatively in cabbage. The highest mean value was observed in T₀, T₂ and T₃, while

lowest mean value occur in T_4 . This could be that at these doses of fertilizer and goat droppings, the production of this phytochemical decline due to the chemicals contained in them.

4. CONCLUSION

Cultivation of vegetables is on the rise due to the increase in demand as a result of the enormous nutrients supplied. This facilitated the interest of many farmers in the cultivation of vegetables, with cabbage being one of the commonly cultivated. The use of chemical fertilizer for vegetable cultivation seems to be the normal practice by farmers due to its quick result in growth enhancement. However many report have indicated that the use of chemical fertilizer could have a residual effect on the nutritional content. This work examined the effect of cabbage cultivation with different organic manure and inorganic fertilizer on the growth and yield parameters and the phytochemical constituents. The result shows that cabbage should better be cultivated with organic manure as manure enhances the growth parameters and also improved the medicinal properties in cabbage in term of its phytochemical content. Further studies are however encouraged to know the constituents of these organic manure which make them to have better growth parameter and more phytochemicals.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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