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Article

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Changes in Morphological, Biochemical and Yield Parameters of *Abelmoschus esculents* (L.) Moench due to Panchagavya Spray

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Abstract: A field experiment was conducted to find the variation in growth, biochemical and yield parameters of black gram under different concentrations (control, 1, 3, 5, 7.5 and 10%) of panchagavya, and all the parameters were increased in 3% concentration. Since there was increase in growth and yield at low concentration of panchagavya, it is recommended that the panchagavya can be used for spray after diluted properly.

Keywords: black gram; panchagavya; biochemical parameter; morphology; yield; growth.

1. Introduction

In Veda, cow's urine was compared to the nectar. In substrata several medicinal properties of cow's urine have been mentioned and are known to cause weight loss, reversal of certain cardiac and kidney problems, indigestion, stomachache, edema, etc. Cow urine has a unique place in Ayurvedha and has been described in Sushrita Sumhita and Ashtanga Sangraha to be most effective substances secretion of animal origin with innumerable therapeutic values. It has been recognized as water of life or Amrita (beverages of immortality) the nectar of the God. In India drinking of cow urine has been practiced for thousands of years. Panchagavya is a term used in Ayurveda to describe five important substances obtained from cow namely urine, dung, milk, ghee and curd. A number of formulations

mentioned in Ayurveda described the use of panchagavya components either alone *or* in combination with drugs of herbal, animal or mineral origin (Shah, 1997).

Green revolution had lead to intensify agriculture to meet the ever increasing demand for food and fibre, which is a practice at great cost to the environment resulting in continuous lose of natural ecosystem, ground water, food staff pollution and other environmental degradation (Gupta and Gopal, 2001). The indiscriminate use of chemical pesticides in modern agriculture resulted in the development of several problems such as pesticide resistant insects, resurgences of target and non target pests, destruction of beneficial organism like honey bee, pollinators, parasitors, and predators and pesticide residues in food, and fodder. The awareness about the health and environmental problems due to the continuous use of pesticides resulted in the development of integrated pest management (ipm) and organic farming (Thomas et al., 2001; Prabu, 2004).

Heavy use of chemicals in agriculture has weakened the ecological base addition to degradation of soil, water resources and quality of food. At this juncture a keen awareness has sprung of the adoption of "organic farming" as a remedy to cure. Organic agriculture is low cost and chemical free fertilizers. It is very essential to development a strong workable and compatible package of nutrient management through organic resources for various crops based on scientific facts, local conditions and economic viability (Kannaiyan, 2000).

The current global scenario firmly emphasizes the need to adult eco-friendly agricultural practices for sustainable agriculture. Chemical agriculture has made an adverse impact of the health-care of not only soil but also the beneficial soil microbial communities and the plants cultivated in these soil. This eventually has lead to a high demand organic produce by the present day health conscious society and sporadic attempts are being made by farmers all over the world to detoxify. The land by switching over to organic farming dispenses with chemical fertilizers, pesticides, fungicides and herbicides. In India, organic farming was a well developed and systematized agricultural practice during the past and this "ancient wisdom" obtained through Indian knowledge systems such as Vedas, specify the use of panchagavya in agriculture.

Panchagavya is a foliar nutrition prepared by organic growers of Tamilnadu and widely used for various agricultural and horticultural crops. In Sanskrit, panchagavya means a combination of five products obtained from cow. When suitably mixed and used, these have miraculous effects. In the present study a preliminary attempt has been made to find out the effect of panchagavya spray on the growth, biochemical and yield parameters of *Abelmoschus esculentus* (L.) Moench.

2. Materials and Methods

2.1. Seed Collection

The seeds of *Abelmoschus esculentus* (L.) Moench were collected from the market at Mayiladuthurai, Tamil Nadu.

2.2. Preparation of Panchagavya

Cow dung	1 kg
Cow urine	750 mL
Cow milk	400 mL
Curd milk	400 mL
Ghee	200 mL
Distilled water	10 L

The above mentioned components were mixed thoroughly with ten liter of water, and daily thoroughly mixed with stump on the solution morning and evening, after ten days to make different concentrations (control, 1, 3, 5, 7.5 and 10%) and spray on crops separately.

Field experiment was carried out to assess panchagavya foliar spray and also to arrive at the suitable dilution factor to change the growth and yield of *Abelmoschus esculentus* (L.) Moench. The field experiment was conducted during January 2013 to March 2013 at botanical garden, Department of Botany, A.V.C. College (Autonomous), Mannampandal, Tamil Nadu.

2.3. Design of the Experiment

Experiment Period: Jan 2013 to March 2013;
Experiment Design: Randomised Block Design;
Plot Size: 2 × 2 m;
Crop Studied: *Abelmoschus esculentus* (L.) Moench;
Treatment: Control × Different Concentration of Panchagavya;
Panchagavya Concentration : 1%, 3%, 5%, 7.5% & 10%.

2.4. Morphological Studies

The morphological studies were observed in *Abelmoschus esculentus* (L.) Moench, and plant height (centimeter scale), number of leaves, fresh weight and dry weight (electrical single pan balance) were measured in various concentrations with various intervals (seedling, flowering and yielding).

2.5. Yield Parameters Studies

The yield parameters studies observed in *Abelmoschus esculentus* (L.) Moench on number of fruits and fruit weight (electrical single pan balance) were measured in various concentrations in yielding stage.

2.6. Biochemical Analysis

The biochemical contents (chlorophyll, carotenoid, starch, protein, amino acid and sugars) were analyzed at various stages (seedling, flowering and yielding) of *Abelmoschus esculentus* (L.) Moench.

2.6.1. Chlorophyll

Five hundred milligram of fresh leaf was ground in a mortar and pestle with 10 mL of 80% acetone. The homogenate was centrifuged at 800 g for 15 min. The supernatant was saved. The pellets were extracted with 10 mL of 80% acetone. The supernatant was saved and utilized for chlorophyll estimation. Absorbance was read at 645 nm and 663 nm in Spectronic - 20 (Arnon, 1949).

Chlorophyll a (mg/L) = $(0.0127) \times (O.D. 663) - (0.00269) \times (O.D. 645)$.

Chlorophyll b (mg/L) = $(0.0229) \times (O.D. 645) - (0.00468) \times (O.D. 663)$.

Total chlorophyll (mg/L) = $(0.0202) \times (O.D. 645) + (0.00802) \times (O.D. 663)$.

2.6.2. Carotenoids

The chlorophyll extract was read at 480 nm for quantitative determination of carotenoids. The amount of carotenoids present in the extract was calculated by using the following formula (Kirk and Allen, 1965).

Carotenoids (mg/L) = O.D. 480 - $(0.114 \times O.D. 663)$ - (0.0636 - O.D. 645).

2.6.3. Estimation of protein

(1) Preparation of reagents

Reagent A: 0.4 g of sodium hydroxide was dissolved in 100 mL of distilled water. To this solution, 2 g of sodium carbonate was added.

Reagent B: One per cent of copper sulphate was mixed with equal volume of 2% sodium potassium tartarate.

Reagent C: Fifty milliliter of reagent A and one milliliter of reagent B were taken and mixed freshly at the time of experiment.

Folin - Phenol reagent: One milliliter of Folin - Phenol reagent was diluted with 2 mL of distilled water.

(2) Extraction

Five hundred milligram of plant materials were weighed and macerated in a pestle and mortar with 10 mL of 20% trichloro acetic acid. The homogenate was centrifuged for 15 min at 600 g. The supernatant was discarded. To the pellet, 5 mL of 0.1 N NaOH was added and centrifuged for 5 min. The supernatant was saved and made upto 10 mL of 0.1 N NaOH. This extract was used for protein estimation (Lowry *et al.*, 1951).

(3) Estimation

One milliliter of the extract was taken in a 10 mL test tube and 5 mL of reagent 'C was added. The solution was mixed and kept in darkness for 10 min. Later, 0.5 mL of Folin-Phenol reagent was added and the mixture was kept in dark for 30 min. The sample was read at 660 nm in a UV-Spectrophotometer.

2.6.4. Estimation of amino acids

(1) Ninhydrin reagent

Eight hundred gram of hydrated stannous chloride was dissolved in 500 mL of citrate buffer at pH 5.0 and 20 g of recrystallized Ninhydrin in 500 mL of methyl cellosolve. Then these two solutions were mixed.

(2) Extraction

Five hundred milligram of plant materials were weighed and macerated with a pestle and mortar with 10 mL of 80% ethanol. The homogenate was centrifuged for 10 min at 800 g. The supernatant was saved. The extract was used for the estimation of amino acids (Moore and Stein, 1948).

(3) Estimation

One milliliter of the extract was pipetted out into a test tube. A drop of methyl red indicator was added. The sample was neutralized with 1 mL of 0.1 N sodium hydroxide. To this, 1 mL of Ninhydrin reagent was added and mixed thoroughly. The content of the test tube was heated for 20 min in a boiling water bath. Five milliliter of the diluent solution was added and heated in water bath for 10 min. The tubes were cooled under the running water and the contents were mixed thoroughly. Blank was prepared with 1 mL of distilled water or ethanol. The absorbance was read at 570 nm in a UV - Spectrophotometer.

2.6.5. Estimation of sugars

(1) Preparation of reagents

Reagent A: Twenty five gram of anhydrous sodium carbonate, 25 gram of sodium potassium tartarate, 20 gram of sodium bicarbonate and 200 grams of anhydrous sodium sulphate were dissolved in 800 mL of distilled water and made upto 1000 mL. Then, it was filtered and stored in a glass stoppered brown bottle.

Reagent B: Fifteen per cent copper sulphate containing 1 or 2 drops of concentrated sulphuric acid.

Reagent C: Fifty milliliter of reagent A and one milliliter of reagent B were mixed and it was prepared freshly at the time of experiment.

Arsenomolybdate reagent: To 450 mL of distilled water, 25 g ammonium molybdate, 21 mL of concentrated sulphuric acid were added and 3 g of sodium arsenate was dissolved in 25 mL of distilled water. The mixture was kept in a water bath at 37 °C for 24 to 58 hours. The reagent was stored in a glass stoppered brown bottle.

(2) Extraction

Five hundred milligram of plant materials were weighed and the homogenate was centrifuged for 10 min at 800 g. The supernatant was saved. Then, the ethanol was evaporated in a water bath at 50 °C. The net content was made upto 20 mL with distilled water and the extract was used for the estimation of total sugar (Nelson, 1944).

(3) Estimation

One milliliter of extract was taken in a 25 mL marked test tube. The 1 mL of regent 'C was added. Then, the mixture was heated for 20 min at 100 °C in a boiling water bath, and cooled and 1 mL of arsenomolybdate reagent was added. The solution was thoroughly mixed and diluted to 25 mL with distilled water. The sample was read at 520 nm in a UV- spectrophotometer.

2.6.6. Estimation of starch

The ethanol insoluble residue left behind after alcoholic extraction of the original material was taken for the extraction of starch. The residue was dissolved in 6.5 mL of 52% perchloric acid for one hour. It was centrifuged and made upto 100 mL in a volumetric flask with distilled water. One milliliter of this solution was further diluted with 5 mL distilled water. To this, 10 mL freshly prepared anthronc reagent was added. The content was heated for 7 mins at 100 °C in a boiling water bath. The tube was then cooled rapidly, shaken well and the appeared colour was read at 630 nm in Spectronic - 20 (McCready *et al.*, 1950).

3. Results

Field experiment was conducted to know the changes in morphological, biochemical and yield parameters of *Abelmoschus esculents* (L.) Moench due to panchagavya spray. The morphological parameters of *Ablemoscus esculents* at various stages (seedlings, flowering and yielding) are shown in Plate 1 and Table 1.



Panchagavya concentration



Plate 1. Effect of panchagavya spray on Abelmoschus esculentus (L.) Moench

Concentration	Securing Stage					Floweri	ng Stage		Yielding Stage				
of panchagavya	Plant Height (cm/plant)	Fresh Weight (mg/plant)	dry weight (mg/plant)	No. of leaves	Plant Height (cm/plant)	Fresh Weight (mg/plant)	dry weight (mg/plant)	No. of leaves	Plant Height (cm/plant)	Fresh Weight (mg/plant)	dry weight (mg/plant)	No. of leaves	
Control	15.4	20.4	8.4	5.2	50.4	80.2	20.2	9.6	55.6	90.4	21.4	8.2	
1%	16.8	22.6	9.2	6.4	65.6	90.6	22.6	10.2	68.2	92.1	22.6	9.3	
	(9.09)	(10.78)	(9.52)	(23.07)	(30.15)	(12.96)	(11.88)	(6.25)	(22.66)	(1.88)	(5.60)	(13.41)	
3%	19.6	28.6	10.6	8.4	70.7	96.4	24.4	12.6	78.6	96.4	25.3	10.6	
	(27.27)	(40.19)	(26.19)	(61.53)	(40.27)	(20.19)	(20.79)	(31.25)	(41.36)	(6.63)	(18.22)	(29.26)	
5%	14.8	22.1	7.3	7.3	74.6	92.6	21.6	11.4	76.2	91.3	23.6	9.1	
	(3.89)	(8.33)	(13.09)	(42.30)	(48.01)	(15.46)	(6.93)	(18.75)	(37.05)	(0.99)	(10.28)	(10.97)	
7.5%	14.2	20.5	7.2	6.1	60.9	80.3	19.2	9.2	70.3	90.1	22.2	8.3	
	(7.79)	(0.49)	(14.28)	(17.30)	(20.83)	(0.124)	(4.95)	(4.16)	(26.43)	(0.33)	(3.73)	(1.21)	
10%	10.6	19.6	6.6	5.3	68.6	79.6	19.6	9.4	70.1	89.4	20.4	7.9	
	(31.16)	(3.92)	(-21.42)	(1.92)	(36.11)	(0.748)	(2.97)	(2.08)	(26.07)	(1.10)	(4.67)	(3.65)	

Table 1. The morphological parameters of *Abelmoschus esculentus* (L.) Moench grown under different concentrations of panchagavya spray

Note: (\pm) percentage over control is expressed in parenthesis.

The morphological parameters such as plant height, number of leaves, fresh weight, and dry weight of *Abelmoschus esculents* (L.) Moench were increased with the age of the plant. The highest morphological parameters (plant height 19.6, 70.7 and 78.6 cm/plant; the fresh weight 28.6, 96.4 and 96.4 mg/plant; and dry weight 10.6, 24.4 and 25.3 mg/plant on seedling, flowering and yielding stages respectively) of *Abelmoschus esculents* (L.) Moench were recorded in the plants sprayed with 3% concentration of panchagavya when compared with control as well as other concentrations.

The photosynthetic pigments (chl. a, chl. b, and carotenoid) contents estimated at various growth stages of *Abelmoschus esculents* (L.) Moench grown under different concentrations of panchagavya spray are given in Table 2.

The pigment content gradually increased in seedling and flowering, and decreased in yielding stage. The highest pigment contents (chl. a., 0.414, 0.639, and 0.326 mg/plant; chl. b, 0.386, 0.522 and 0.244 mg/plant; carotenoid, 0.326, 0.412 and 0.128 mg/plant on seedling, flowering and yielding stage respectively) of *Abelmoschus esculentus* (L.) Moench were recorded in the 3% concentration of panchagavya spray when compared with control and other concentrations.

The biochemical contents such as protein, starch, amino acid and sugars at various stages of plants sprayed with different concentrations of panchagavya spray is presented in Table 3.

Concentration of	S	Seedling Stage	e	F	lowering Stag	ge	Yielding Stage			
Panchagavya	Chlorophyll a (mg/plant)	Chlorophyll b (mg/plant)	Carotenoid (mg/plant)	Chlorophyll a (mg/plant)	Chlorophyll b (mg/plant)	Carotenoid (mg/plant)	Chlorophyll a (mg/plant)	Chlorophyll b (mg/plant)	Carotenoid (mg/plant)	
Control	0.282	0.216	0.236	0.316	0.312	0.286	0.216	0.192	0.114	
1%	0.312	0.292	0.294	0.426	0.326	0.312	0.263	0.186	0.119	
1 %	(10.638)	(35.185)	(24.576)	(0.348)	(4.487)	(9.090)	(21.75)	(-3.125)	(4.385)	
20/	0.414	0.386	0.326	0.639	0.522	0.412	0.326	0.244	0.128	
3%	(46.808)	(78.703)	(38.135)	(102.21)	(67.30)	(44.05)	(50.92)	(27.08)	(12.28)	
5%	0.310	0.284	0.310	0.522	0.463	0.418	0.263	0.212	0.116	
3%	(9.929)	(31.481)	(31.355)	(65.189)	(48.39)	(46.15)	(21.75)	(10.41)	(1.754)	
7.5%	0.292	0.212	0.263	0.426	0.392	0.374	0.226	0.182	0.112	
7.3%	(3.546)	(-18.51)	(11.440)	(0.348)	(25.64)	(30.76)	(4.62)	(-5.20)	(-1.75)	
10%	0.264	0.236	0.254	0.386	0.366	0.294	0.212	0.186	0.111	
10%	(-6.382)	(9.259)	(7.627)	(22.151)	(17.30)	(2.79)	(-1.85)	(3.125)	(-2.631)	

Table 2. Photosynthetic pigment contents of *Abelmoschus esculentus* (L.) Moench grown under different concentrations of panchagavya spray

Note: (\pm) percentage over control is expressed in parenthesis.

Table 3. Biochemical contents of *Abelmoschus esculentus (L.) Moench* grown under different concentrations of panchagavya spray

Concentration of	Seedling Stage					Flowering Stage				Yielding Stage				
Panchagavya	Protein	Starch	Amino	Sugars	Protein	Starch	Amino	Sugars	Protein	Starch	Amino	Sugars		
	(mg/plant)	(mg/plant)	acid	(mg/plant)	(mg/plant)	(mg/plant)	acid	(mg/plant)	(mg/plant)	(mg/plant)	acid	(mg/plant)		
			(mg/plant)				(mg/plant)				(mg/plant)			
Control	0.362	0.426	0.316	0.386	0.414	0.524	0.412	0.414	0.314	0.364	0.312	0.294		
1%	0.374	0.463	0.326	0.342	0.492	0.592	0.43	0.463	0.363	0.386	0.324	0.312		
1%	(3.314)	(8.685)	(3.164)	(-11.39)	(16.03)	(12.97)	(12.37)	(11.83)	(15.60)	(6.04)	(3.84)	(6.12)		
3%	0.414	0.512	0.384	0.414	0.526	0.642	0.514	0.472	0.414	0.418	0.392	0.326		
3%	(14.36)	(20.187)	(21.51)	(7.253)	(24.05)	(22.51)	(19.84)	(14.00)	(31.84)	(14.83)	(25.64)	(10.88)		
5%	0.363	0.492	0.318	0.363	0.414	0.546	0.426	0.386	0.528	0.392	0.317	0.274		
J 70	(0.276)	(15.492)	(0.632)	(-5.95)	(-0.023)	(4.198)	(3.39)	(-6.76)	(4.458)	(7.69)	(16.02)	(-6.80)		
7.5%	0.355	0.416	0.286	0.312	0.372	0.528	0.392	0.344	0.310	0.12	0.315	0.216		
7.5%	(-2.486)	(-2.34)	(-9.493)	(-19.17)	(-12.26)	(0.763)	(-4.85)	(-16.90)	(-1.27)	(-14.28)	(0.961)	(-26.5)		
10%	0.343	0.412	0.263	0.31	0.318	0.512	0.376	0.338	0.286	0.293	0.284	0.212		
1070	(-5.248)	(-3.28)	(-16.77)	(-19.43)	(-25.00)	(-2.29)	(-8.73)	(-18.35)	(-8.91)	(-19.50)	(-8.97)	(-27.89)		

Note: (\pm) percentage over control is expressed in parenthesis.

The plants showed increasing trend in biochemical contents up to flowering stages, and decreased in yielding stage. The highest biochemical contents (protein 0.414, 0.526 and 0.414 mg/plant; starch 0.512, 0.642 and 0.418 mg/plant; and amino acid 0.384, 0.514 and 0.392 mg/plant; sugars 0.414, 0.472 and 0.326 mg/plant) on seedling, flowering and yielding stages of *Abelmoschus esculentus* (L.) Moench were recorded in the plants sprayed with 3% concentration of panchagavya when compared with control and other concentrations.

The yield parameters such as number of fruit and fruit weight of *Abelmoschus esculentus* (L.) Moench sprayed with different concentrations of panchagavya is presented in Table 4. The highest yield parameters such as number of fruit (20), and fruit weight (45 mg/fruit) of *Abelmoschus esculentus* (L.) Moench were recorded in the plants sprayed with 3% concentration of panchagavya when compared with control as well as other concentrations.

C	lonc	entrati	on		No. of Fru	uit]	Fruit W	eight		
concen	trati	ions of j	panchagavya	ı spr	ay							
Table	4 .	Yield	parameters	of	Abelmoschus	esculentus	(L.)	Moench	grown	under	different	

Concentration	No. of Fruit	Fruit Weight (mg/fruit)
Control	12	30
1%	15 (25.0)	40 (33.33)
3%	20 (66.66)	45 (50.0)
5%	18 (50.0)	43 (43.33)
7.5%	16 (33.33)	42 (40.0)
10%	15 (25.0)	28 (-6.66)

Note: (\pm) percentage over control is expressed in parenthesis.

4. Discussion

Many advanced countries mainly depend upon the dairy byproducts because of their commercial, agricultural and medicinal activities play a vital role in the development of the countries. When a new house or building or even a temple constructed in India, the first to enter premises would be the cow because this is considered to be auspicious. In recent years the people have recognized a number of commercial, medicinal and agricultural values from the various products of dairy forms. Tharun *et al.* (1983) have carried out extensive works in this aspects and the environmental management in developing countries. The number of new methods of recycling and controlling measures of organic waste in urban and rural habits was proposed by Furedy (1987) and Blumantal *et al.* (1989). The present study revealed the morphological growth, biochemical and yield parameters of *Abelmoschus esculentus* (L.) Moench grown under the different concentrations of (control, 1, 3, 5, 7.5 and 10%) of panchagavya spray.

The morphological parameters such as plant height, number of leaves, fresh weight and dry weight of *Abelmoschus esculentus* (L.) Moench were increased in 3% panchagavya spray when compared with control and other concentrations. Similar finding were observed in black gram (Swaminathan *et al.*, 2007) and *Coleus forskohili* (Kanimozhi, 2004).

Xu (2001) reported that effective micro organism (EMO) cultures in panchagavya could synthesize phytohormones i.e., auxins and other growth regulators that stimulated plant growth. Chemotrophs and autotropic (ammonifers and nitrifers) present in panchagavya which colonize in the leaves increase the ammonia uptake and enhance total nitrogen supply (Papen, 2002).

The photosynthetic pigments content such as chl. a, chl. b, total chl. and carotenoid of *Abelmoschus esculentus* (L.) Moench were increased in 3% panchagavya spray and decreased in control and other concentration. Similar findings were observed in *Arachis hypogaea* (Subramaniyan, 2005) and *Vigna radiata, Vigna mungo* and *Oryza* sativa (Tharmaraj, 2011).

The biochemical contents (carbohydrates, protein and amino acids) of *Abelmoschus esculentus* (L.) Moench were increased in 3% concentration of panchagavya spray when compared with control and other concentrations. Similar findings were observed in grains of *Oryza sativa* (Chopra and Kunvar, 1976) and *Vigna mu*ngo (Rajasekaran and Balakrishnan, 2002).

The yield parameters (number of fruit, and fruit weight of *Abelmoschus esculentus* (L.) Moench) were increased in 3% panchagavya spray when compared with control and other concentration. Similar findings were observed in *Vigna mungo* and *Oryza sativa* (Rajasekaran and Balakrishanan, 2002), black gram and green gram (Brito and Girija, 2006) and groundnut (Ravikumar, 2012)

The plant growth substances in panchagavya help to bring rapid changes in phenotypes of plants and also improve the growth and productivity (Tharmaraj, 2011).

In panchagavya spray, the nutrients easy transfer to plant through foliar spray and the quantities of IAA and GA present in panchagavya (Kunnal, 1997, Ravikumar, 2012)

5. Conclusions

The present research work was carried out to study the effect of panchagavya spray on growth, yield and biochemical changes of *Abelmoschus esculentus* (L.) Moench. All parameters were increased in 3% concentration. Since there was increase in growth and yield at low concentration of panchagavya, it is recommended that the panchagavya can be used for spray after diluted properly. Cow's urine provides nitrogen which is essential for crop growth. Milk provides protein, fat, carbohydrates, amino acid and calcium. Curd provides lactobacillus which act as a catalyst in the digestion of organic waste. Ghee provides vitamins A and B, calcium and fat. These contents stimulate the growth and yield of all vegetable crops.

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