Section A-Research paper

EFFECT OF ORGANIC MANURES AND PLANT EXTRACT ON NUTRITIONAL VALUES AND YIELD OF CHICK PEA (*CICER ARIETINUM*)

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ABSTRACT

Chickpea, a second most important leguminous food grain cultivated mainly as the rainfed crop in Telangana state. Among other pulses, chickpea is the major source of dietary protein in Indians diet. In the continuation of Effect of Organic Manures and Plant Extracts on Pest Incidence, Growth and Yield of Chickpea, *Cicer arietinum L*, we explored the Nutritional levels in chickpea in different conditions. The highest plant height 60.493 cm was observed in T_1-Vermicompost (10 t/ha) + NSKE (5%) followed by T_6 - Poultry manure (5 t/ha) + chilli garlic extract (100 gm/lit) with 57.963 which was on par with T_2 -Vermicompost (10 t/ha) + chilli garlic extract (100 gm/lit), T_3 -Vermicompost (10 t/ha) + tobacco leaf decoction (5% W/v), T_4 -Vermicompost (10 t/ha) + pongamia seed extract (5% W/v), T_5 - Poultry manure (5 t/ha) + NSKE (5%). The more branches per plant 7.567 no. was recorded in T_1 which was on par with T_8 - Poultry manure (5 t/ha) + pongamia seed extract (5% W/v) with 7.620 no. followed by T_5 - Poultry manure (5 t/ha) + NSKE (5%).T_1-Vermicompost (10 t/ha) + NSKE (5%)recorded highest i.e. 69.94 no. of pods per plant with 1569.45 kg/ha grain yield and it was significantly different from other treatments followed by T_5 - Poultry manure (5 t/ha) + NSKE (5%).The T1 has greater values of protein (20.47 g), fat (6.04 g), carbohydrate (62.95 g), fiber (12.2 g), sugar (10.78 g), Ca (57.56 mg) and Fe (4.37 mg) and next highest nutritional values recorded T_5- Poultry manure (5 t/ha) + NSKE (5%). Highest Benefit cost ratio was recorded in T_1 and it was significantly superior over all the treatments.

Keywords: Helicoverpa armigera, Spodoptera exigua, Chickpea, Cicer arietinum, RBD Method.

1. Introduction.

Conventional agricultural systems have been characterized by high input of chemical fertilizer and pesticides leading to soil health deterioration and poor product quality due to a reduction in soil organic matter content. Continuous addition of organic matter to soil is necessary to ensure a balanced supply of plant nutrients (Gupta et al. 2014). In recent years, organic farming has gained attention as it maintains soil health, prevents soil erosion, uses natural pest controlling agents and farm products have better nutritive quality. It is evident

from the available literature that organic manure contains significant quantities of NPK (Nitrogen phosphorous and potassium). However, organic manure directly be applied to the agricultural fields since these may not cause any phytotoxicity or may not destroy the natural fertility of the soil. Therefore, their stabilization is very appropriate to their application in agricultural fields (Gupta et al. 2014). Vermicomposting of non-toxic biodegradable matter produces stabilized humus like product known as vermicompost, which has a great potential as soil amendment (Arancon et al. 2003). Vermicompost is a very good soil conditioner that is rich in NPK, micronutrients and growth hormones. Vermicompost application to soil also increases microbial populations and activities that further influence nutrient cycling, production of plant growth-regulating materials and build up plant resistance or tolerance to pathogen and nematode attacks (Gopalakrishnan et al. 2011). Organic amendments application enhances soil microbial activities as compared to mineral fertilizer and unamended treatments (Gopinath et al. 2008). Ferreras et al. (2006) reported that soil structure stability, organic carbon and microbial activity in soil were improved after two applications of vermicomposts. Jouquet et al. (2010) reported that both compost and vermicompost led to an improvement in soil properties with an increase in the pH, soil organic matter and nutrient content, compared to soil fertilized with chemical fertilizers. Khan et al. (2015) reported that application of vermicompost caused a substantial increase (54–83 %) in the soil organic carbon pool of the soil. Chickpea (Cicer arietinum L.) is an important food legume, which ranks third among the world's pulse crops, and is grown in more than 50 countries (89.7 % area in Asia, 4.3 % in Africa, 2.6 % in Oceania, 2.9 % in Americas and 0.4 % in Europe) (Lake and Sadras 2014). India is the largest chickpea producing country accounting for 67 % (8.8 million metric tonnes) (FAO 2014). Chickpea is also a vital source of protein for millions of people in India and other developing countries, predominantly in South Asia, who are largely vegetarian. In addition to having high protein content (20–22 %), it is rich in fiber, P, Ca, Mg, Fe, Cu and b-carotene. Farmers have notion that chickpea, being a legume crop, does not need nutrients and usually cultivate it on marginal lands without applying chemical fertilizer. The yield gap of chickpea can be abridged, by adopting the advanced production technology accompanying with use of inoculums, balanced nutrition, weed management and high yielding varieties (Hakoomat et al. 2004). A literature survey has indicated that vermicompost produced from different wastes has different physicochemical properties and in turn has different effects on the crops' growth and yield. Based on this it is hypothesized that vermicompost with different NPK contents have different effects on crops and results of a vermicompost on a crop cannot be generalized. Keeping this view effect of vermicompost and natural products on growth and productivity of chickpea plants has been investigated.

2. Methodology.

2.1. Study the effect of organic manure and plant extracts on nutritional values of chick pea.

In above field experiments, to correlate non- pesticidal management with organic manures and plant extracts on nutritional value of chickpea, samples were collected from the all the treatments and sent to Quality Control Laboratory, Professor Jayashankar Telangana state

agricultural university, Rajendrenagar for evaluating nutritional values of chick pea (100 gm) like Protein (Biuret method) , fat (Bligh, E.G. and W.J. Dyer, 1959) , fiber (Maynard, A.J. 1970), (sugar Anthrone method, 1962), Calcium (Titrimetric Determination) and iron(Jackson, 1973).The data were compared to know the which treatment is influencing the nutritional values were drawn.

2.2. Estimation of Protein - Biuret method (Layne, E. 1957). **Reagents.**

Biuret reagent: Dissolve 3 g of $CuSO_4$. $5H_2O$ and 9g of sodium potassium tartrate in 500 ml of 0.2N NaOH solution. To this solution, add 5g of potassium iodide and make up to 1 litre with 0.2 N NaOH. Standard protein solution: 5mg of Bovine Serum Albumin/ml water. Prepare fresh. **Procedure.** Make up 1 ml of sample to 4 ml with water. Add 6 ml of biuret reagent and mix well. Keep at 37°C for 10 min.Cool and read the absorbance at 520 nm (green filter) against a reagent blank (prepare similarly with 4ml of water).Draw the standard graph by pipetting out 0.1, 0.2, 0.4, 0.6, 0.8 and 1.0 ml of standard protein solution into a series of test tubes and making up the volume in each to 4ml with water. Carry out steps 2 to 4. Calculate the protein content in the sample using a standard graph. In order to overcome interfering substances, a modified biuret method may be followed. For this, make up the volume of sample to 1 ml with water. Add 1ml of 10% TCA, mix well and centrifuge at 3000 rpm for 10 min. To the precipitate, add 2ml of ethyl ether mix and recentrifuged. Dissolve the final dry precipitate in 4ml of water, mix and carry out steps from 2 to 6.

2.3. **Estimation of fats (Bligh and Dyer. 1959).**

Reagents. Chloroform: methanol, (10:20,v/v).Chloroform.Procedure.Homogenize about 5 g material in a blender for two minutes in a mixture of chloroform: methanol. Add 10 ml of chloroform and homogenize for a minute. Add 10ml water and homogenize further for a minute. Filter and wash the precipitate with 10 ml of chloroform, refilter and transfer to a separating funnel. Remove the upper methanol-water layer by aspiration and a small volume of the chloroform layer is also removed to ensure complete removal of the upper layer. Record again the volume of chloroform layer ('y' ml), transfer quantitatively into a pre-weighed conical flask ('a' g). Evaporate in a 40-50° C water bath. Cool and dry the residue over phosphoric anhydride in vacuum desiccators. Weigh the flask second time ('b' g.). Add 10 ml of chloroform 3 times to dissolve the lipids. Evaporate and dry the flask as in steps 9 and 10. Weigh the flask a third time ('c’ g).

2.4. **Estimation of crude fiber** (Maynard, A.J. 1970)**.**

Sulphuric acid solution: 1.25g concentrated sulphuric acid diluted to 100ml (concentration must be checked by titration). Sodium hydroxide solution: 1.25g sodium hydroxide in 100 ml distilled water (concentration must be checked by titration with standard acid). Extract 2g of ground sample with ether or petroleum ether to remove fat (initial boiling temperature 35-38°C and final temperature, 52°C). If fat content is less than 1% extraction may be omitted. Boil 2g of dried sample with 200 ml of H_2SO_4 for 30 min with bumping chips. Filter through muslin cloth and wash with boiling water until washings are free of acid. Boil the residue

with 200 ml of NaOH for 30 min. Filter through muslin cloth again and wash with 25 ml of boiling H_2SO_4, three 50 ml portions of water and 25 ml of alcohol. Remove the residue and transfer to pre-weighed ashing dish (W_1g). Dry the residue for 2h at 130 + 2°C. Cool the dish in a desiccator and weigh (W_2 g). Ignite for 30 min at 600 + 15°C.Cool in a desiccator and reweigh (W_3g).

2.5. **Estimation of total sugars-Anthrone method (**Hedge and Hofreiter. 1962)**.**

2.5N HCL. Anthrone reagent: Dissolve 200mg anthrone in 100ml of ice cold 95% H_2SO_4 prepare fresh before use. Standard glucose(stock): Dissolve 100mg in 100ml water. Working standard: 10ml of stock diluted to 100ml with distilled water. Store refrigerated after adding a few drops of toluene. Weigh 100mg of the sample into a boiling tube. Hydrolyze by keeping it in a boiling water bath for 3 hours with 5ml of 2.5N HCL and cool to room temperature. Neutralize it with solid sodium carbonate until the effervescence ceases. Makeup the volume to 100 ml and centrifuge. Collect the supernatant and take 0.5 and 1ml aliquots for analysis. Prepare the standards by taking 0, 0.2, 0.4, 0.6, 0.8 and 1mlof the working standard. '0' serves as blank. Make up the volume to 1ml in all the tubes including the sample tubes by adding distilled water. Then add 4ml of anthrone reagent. Heat for 8 min in a boiling water bath. Cool rapidly and read the green to dark green colour at 630 nm. Draw a standard graph by plotting concentration of the standard on the X-axis versus absorbance on the Y-axis. From the graph calculate the amount of carbohydrates present in the sample tube.

2.6. Estimation of Protein - Biuret method (Layne, E. 1957)**.**

Biuret reagent: Dissolve 3 g of $CuSO_4$. $5H_2O$ and 9g of sodium potassium tartarate in 500 ml of 0.2N NaOH solution. To this solution, add 5g of potassium iodide and make up to 1 litre with 0.2 N NaOH. Standard protein solution: 5mg of Bovine Serum Albumin/ml water. Prepare fresh. Make up 1 ml of sample to 4 ml with water. Add 6 ml of biuret reagent and mix well. Keep at 37°C for 10 min.Cool and read the absorbance at 520 nm (green filter) against a reagent blank (prepare similarly with 4ml of water). Draw the standard graph by pipetting out 0.1, 0.2, 0.4, 0.6, 0.8 and 1.0 ml of standard protein solution into a series of test tubes and making up the volume in each to 4ml with water. Carry out steps 2 to 4. Calculate the protein content in the sample using a standard graph. In order to overcome interfering substances, a modified biuret method may be followed. For this, make up the volume of sample to 1 ml with water. Add 1ml of 10% TCA, mix well and centrifuge at 3000 rpm for 10 min. To the precipitate, add 2ml of ethyl ether mix and recentrifuge. Dissolve the final dry precipitate in 4ml of water, mix and carry out steps from 2 to 6.

2.7. **Determination of Calcium by titrimetry (**Lawani *et al*., 2014)**.**

A 25.0 mL aliquot of each digest was pipetted into a beaker and 1M NaOH solution was added to adjust the pH to to 12-13. Two drops of solochrome dark blue were then added and immediately titrated against a 0.01M EDTA 12-13. Two drops of solochrome dark blue was then added and immediately titrated against a 0.01M EDTA solution to the blue end-point.

For every determination and evaluation, a standard curve of mass of calcium (mg) versus amount of EDTA (millimoles) was plotted.

2.8. Estimation of Iron (Jackson, 1973).

Ten ml of the plant extract (triple acid extract) was taken in a 250 ml beaker and added 0.5 ml of concentrated HCl. Two ml of hydroxylamine hydrochloride and one ml or ortho phenanthroline, pH adjusted between 1.3 - 2.7 were added and made up to 25 ml. The colour developed was measured in Systronic colorimeter using 490 m. This was compared against the standard.

2.9. Economics of organic manures and plant extracts use in chickpea.

Benefit cost ratio was worked out in all the treatments by following cost constants of each parameter as follows. Cost of treatment (Rs/ha): Cost of insecticide, labourer wages and sprayer hiring charges were summed up to work out the cost of respective treatment. Cost of insecticide (Rs/ha): Insecticides were purchased from local market and the cost of insecticide was obtained by multiplying total quantity (kg/lit.) of respective pesticide required (for per hectare application) with the prevalent market price (Rs.) for per lit./kg of respective pesticide.

Laborer Wages (Rs/ha): Two laborers were considered sufficient for spraying in a day over one hectare crop Sprayer hiring charges (Rs/ha): The hire charge of power sprayer was considered as Rs.4000.00 per hectare (including the petrol fuel cost) for respective treatments for 3 sprays. Additional yield (Q/ha): This was obtained by subtracting the values of control yield from total fruit yield of a respective treatment. Gross returns (Rs./ha): It was calculated by multiplying the yield with rate per quintal over the untreated control . Cost of cultivation and cost of treatment imposition was deducted from the gross returns, to find out net returns.Net return (Rs/ha): This was calculated separately by subtracting the cost of treatment from additional income of respective treatment. Benefit- cost ratio: This was calculated separately for each treatment as per following formulae.

3. Results and Discussion.

3.1. Effect of organic manures and plant extracts on nutritional values of chickpea.

Effect of organic manures and plant extracts on nutritional values of chickpea was presented in Table.1 and all the treatments were shown great improvement in increasing nutritional value of chickpea over control and standard check. The T_1 has greater values of protein (20.47 g), fat (6.04 g), carbohydrate (62.95 g), fiber (12.2 g), sugar (10.78 g), Ca (57.56 mg) and Fe (4.37 mg) and next highest nutritional values recorded T_5- Poultry manure (5 t/ha) + NSKE (5%). It is clearly evident that, vermicompost with NSKE and poulty manure with NSKE not only help in reducing the insect population besides improving growth and yield in chickpea but also improved the nutritional values of chickpea. The next best treatments were T_7- Poultry manure (5 t/ha) + tobacco leaf decotion (5% W/v), T_8- Poultry manure (5 t/ha) + pongamia seed extract (5% W/v), T_6- Poultry manure (5 t/ha) + chilli garlic

extract (100 gm/lit), whereas, moderate nutritional values were observed t2, t3 and T_4- Vermicompost (10 t/ha) + pongamia seed extract (5% W/v).The lowest nutritional values recorded in T_9 - RDN (25 kg N, 125kg P & 125 kg K/Ha) + spinosad (0.3 ml/lit) with protein (17.96g), fat (5.12 g), carbohydrate (57.69 g), fiber (12.56 g), sugar (7.56 g), Ca (52.1 mg) and Fe (3.25 mg).The above results are coincide with the findings of Chibbar *et al*, 2010 and Geervani, 1991 who reported that chickpea is a good source of carbohydrates and protein, together constituting about 80% of the total dry seed mass in comparison to other pulses. Muhammad Imran Khan *et al*., 2021 findings suggest that the co-addition of organic manures and P fertilizers along with plant growth promoting bacteria (i.e., Bacillus sp. MN-54) not only increases the growth and yield but also improves nodulation, nutrient uptake, and crude proteins content in chickpea. In case of chickpea, values for crude protein, crude fat, crude fiber and vitamin B_1 contents in organically grown chickpea samples were comparatively towards higher side with per cent difference of +21.38, 17.24, +33.72 and +9.09 respectively (Rameshwar *et al*.,2017).

Table. 1. Effect of organic manures and plant extracts on nutritional values of chickpea

Treatment No.	Particulars	Nutrional values for 100gm						
		Protein (g)	Fat (g)	Carbohydrates (g)	Fiber (g)	Sugar (g)	Calcium (mg)	Iron (mg)
T_1	Vermicompost (10 t/ha) + NSKE (5%)	20.47	6.04	62.95	12.2	10.78	57.56	4.37
T_2	Vermicompost(10 t/ha) + chilli garlic extract (100 gm/lit)	18.45	5.89	58.45	10.28	9.85	54.27	4.01
T_3	Vermicompost(10 t/ha) + tobacco leaf decotion (5% W/v)	18.43	5.68	58.35	10.56	9.45	54.38	4.12
T_4	Vermicompost (10 t/ha)+ pongamia seed extract (5% W/v)	17.56	5.45	58.65	10.85	9.68	54.25	4.01
T_5	Poultry manure (5t/ha)+ NSKE (5%)	20.34	6.12	61.89	12.65	10.85	57.82	4.39
T_6	Poultry manure (5t/ha) + chilli	18.65	5.78	57.98	1.056	9.56	54.65	4.01

	garlic extract (100 gm/lit)							
T_7	Poultry manure (5t/ha) + tobacco leaf decotion(5% W/v)	18.78	5.48	58.65	10.56	9.47	54.85	4.25
T_8	Poultry manure (5t/ha) + pongamia seed extract (5% W/v)	17.68	5.01	57.85	10.75	9.58	53.45	4.36
T_9	Recommended dose of fertilizers (25 kg N, 125kg P & 125 kg K/Ha) + spinosad (0.3 ml/lit)	17.96	5.12	57.69	12.56	7.56	52.1	3.25
T_{10}	Control	15.74	4.89	54.23	10.25	7.12	50.25	3.12

3.2. Economics of organic manures and plant extracts use in chickpea.

The Benefit cost ratio among selected treatments is presented in the Table 2 to 4 and exhibited a range of 1.73 to 3.33. All the treatments were found statistically superior to untreated control. However, among all the treatment the highest Benefit cost ratio was recorded in T_1 It was significantly superior over all the treatments. It was followed by T_5, T_2, T_3, T_4. The treatment 7 and 8 recorded same 2.85 benefit cost ration and lowest Benefit cost ratio was recorded in T_9 (2.55), followed by T_{10}- control (1.73). The present results are in line with Bhushan *et al.,* 2011 who reported that Neem Seed Kernel Extract (NSKE 5 %) was found most effective in reducing the larval population and pod damage. Yield and Cost Benefit ratio (C: B ratio) was also found maximum in the NSKE treated plots. The application of vermicompost (2 t/ha) recorded the higher cost of cultivation as well as maximum gross return than the other treatments (Singh *et al.,* 2014). Determination of Calcium by titrimetry. 25.0 mL aliquot of each digest was pipetted into a beaker and 1M NaOH solution was added to adjust the pH.

Table.2.General cost of different farm activities of chickpea cultivation (Rs per Ha): (Excluding the cost of the treatment inputs)

Sl.No.	Cultural practices	Cost ha^{-1} (Rs)
1	Seeds	2,800.00
2	Field preparation	3,000.00
3	sowing	1,000.00
4	Hired labour	2,000.00
5	Bullock labour	3,000.00
6	Weeding	3000.00
7	Irrigation	3,000.00
8	Harvesting, Threshing, Drying	2,000.00
9	Miscellaneous	2,000.00
	Total	21,800.00

Table.3. Pooled data of Cost of treatments during experimentation period (*Rabi, 2014-15 & 2015-16*).

Treatment No.	Particulars	Rate of insecticide (kg/l)	Labour charges (Rs./Ha)	Total cost of Insecticides and sprayings
T1	Vermicompost (10 t/ha) + NSKE (5%)	2500	4,000	6500
T2	Vermicompost (10 t/ha) + chilli garlic extract (100 gm/lit)	2700	4,000	6700
T3	Vermicompost (10 t/ha) + tobacco leaf decotion (5% W/v)	2900	4,000	6900
T4	Vermicompost (10 t/ha) + pongamia seed extract (5% W/v)	3,000	4,000	7000
T5	Poultry manure (5 t/ha) + NSKE (5%)	2800	4,000	6800
T6	Poultry manure (5 t/ha) + chilli garlic extract (100 gm/lit)	3200	4,000	7200
T7	Poultry manure (5 t/ha) + tobacco leaf decotion (5% W/v)	3350	4,000	7350

T8	**Poultry manure (5 t/ha) + pongamia seed extract (5% W/v)**	**3560**	**4,000**	**7560**
T9	**Recommended dose of fertilizers (25 kg N, 125kg P & 125 kg K/Ha) + spinosad (0.3 ml/lit)**	**6900**	**4000**	**10900**

Table.4. BC Ratio of different treatments during experimentation period.

Treatment No.	**Particulars**	**Yield T/ha**	**Returns (Rs)**	**Cost of cultivation**	**Additional cost of Insecticide and sprayings (Rs)**	**Total cost of cultivation**	**Net profit**	**BCR**
T_1	Vermicompost(10 t/ha) + NSKE (5%)	1569.45	94167	21800	6500	28300	65867	3.33
T_2	Vermicompost(10 t/ha) + chilli garlic extract (100 gm/lit)	1485.5	89130	21800	6700	28500	60630	3.13
T_3	Vermicompost(10 t/ha) + tobacco leaf decotion (5% W/v)	1428.5	85710	21800	6900	28700	57010	2.99
T_4	Vermicompost(10 t/ha)+ pongamia seed extract (5% W/v)	1399.5	83970	21800	7000	28800	55170	2.92
T_5	Poultry manure (5t/ha)+ NSKE (5%)	1564.5	93870	21800	6800	28600	65270	3.28
T_6	Poultry manure (5t/ha) + chilli garlic extract (100 gm/lit)	1488.5	89310	21800	7200	29000	60310	3.08
T_7	Poultry manure (5t/ha) + tobacco leaf decotion(5% W/v)	1386	83160	21800	7350	29150	54010	2.85
T_8	Poultry manure (5t/ha)	1392.5	83550	21800	7560	29360	54190	2.85

	+ pongamia seed extract (5% W/v)							
T_9	Recommended dose of fertilizers (25 kg N, 125kg P & 125 kg K/Ha) + spinosad(0.3 ml/lit)	1388.5	83310	21800	10900	32700	50610	2.55
T_{10}	Control	627.5	37650	21800	0	21800	15850	1.73

4. Conclusion.

The obtained results highlight the significant effects of vermicompost treatment in chickpea variety on plant growth and production, and the hydration properties of seeds. A lower influence of fertilization, instead, was observed on bioactive compounds, and no effect was assessed on the antioxidant activity. The highest seed yields were obtained with vermicompost applied. The highest 100 seed weight (gm) was recorded in the T_1-Vermicompost (10 t/ha) + NSKE (5%) with 20 gm which was on par with T_9 - RDN (25 kg N, 125kg P & 125 kg K/Ha) + spinosad(0.3 ml/lit), T_5- Poultry manure (5 t/ha) + NSKE (5%) with 19.78 and 19.897 respectively. The highest plant height 60.493 cm was observed in T_1-Vermicompost (10 t/ha) + NSKE (5%)followed by T_6 - Poultry manure (5 t/ha) + chilli garlic extract (100 gm/lit) with 57.963 which was on par with T_2 -Vermicompost (10 t/ha) + chilli garlic extract (100 gm/lit), T_3 -Vermicompost (10 t/ha) + tobacco leaf decotion (5% W/v), T_4 -Vermicompost (10 t/ha) + pongamia seed extract (5% W/v), T_5 - Poultry manure (5 t/ha) + NSKE (5%). The more branches per plant 7.567 no. was recorded in T_1 which was on par with T_8 - Poultry manure (5 t/ha) + pongamia seed extract (5% W/v) with 7.620 no. followed by T_5 - Poultry manure (5 t/ha) + NSKE (5%). T_1-Vermicompost (10 t/ha) + NSKE (5%) recorded highest i.e. 69.94 no. of pods per plant with 1569.45 kg/ha grain yield and it was significantly different from other treatments followed by T_5 - Poultry manure (5 t/ha) + NSKE (5%).The T1 has greater values of protein (20.47 g), fat (6.04 g), carbohydrate (62.95 g), fiber (12.2 g), sugar (10.78 g), Ca (57.56 mg) and Fe (4.37 mg) and next highest nutritional values recorded T_5- Poultry manure (5 t/ha) + NSKE (5%). Highest Benefit cost ratio was recorded in T1 It was significantly superior over all the treatments.

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Conflict of interest.

Authors do not have any conflict with others or elsevier.

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solution to the blue end-point. For every determination and evaluation, a standard curve of mass of calcium (mg)
versus amount of EDTA (millimoles) was plotted.

2.3 Determination of Calcium by titrimetry

A 25.0 mL aliquot of each digest was pipetted into a beaker and 1M NaOH solution was added to adjust the pH
to 12-13. Two drops of solochrome dark blue was then added and immediately titrated against a 0.01M EDTA
solution to the blue end-point. For every determination and evaluation, a standard curve of mass of calcium (mg)
versus amount of EDTA (millimoles) was plo