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ORIGINAL RESEARCH

Influence of vermi-fortification on chickpea (*Cicer arietinum L.*) growth and photosynthetic pigments

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Abstract
Purpose The aim of this study was to investigate the effect of different vermicomposts and their dosages on chickpea (*Cicer arietinum* L.) plants in pot culture experiment.
Methods A total of five potting media were prepared containing soil and vermicompost. Soil fortified with 10 and 20 % vermicompost was used as potting media. The fertility status of soil and vermicomposts was quantified. In these potting media, growth, yield and biochemical parameters of chickpea plants were studied up to 90 days.
Results The results showed that the fortification of soil with vermicompost significantly stimulated the chickpea plant growth. The plant height, plant shoot biomass, number of pods and photosynthetic pigments were significantly higher in vermicompost-fortified experiments, whereas vermicompost fortification had no significant effect on chickpea seed germination as it was ≈100 % in all experiments. Total chlorophyll content in chickpea leaves was in the range of 0.437–1.07 mg/g. Similarly, carotenoid content was minimum in control and maximum in 20 % vermicompost containing potting media.
Conclusion It was concluded that if soil is fortified with appropriate quantities of vermicompost, the chickpea production per unit area could be enhanced significantly.

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Keywords Chickpea · Organic manures · Vermicompost · Chlorophyll · Carotenoids · Vermi-fortification

Abbreviations

CD	Cow dung
HC-3	Haryana channa-3
FIS	Food industry sludge
DAS	Days after sowing
TCa	Total calcium
TNa	Total sodium
NPK	Nitrogen phosphorous and potassium
EC	Electric conductivity
TOC	Total organic carbon
TKN	Total Kjeldhal nitrogen
TK	Total potassium
TP	Total phosphorus
CN	Carbon nitrogen ratio

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 fraction of solid wastes contains significant quantities of NPK.

However, organic wastes cannot directly be applied to the agricultural fields since these may cause phytotoxicity or may destroy the natural fertility of the soil. Therefore, their stabilization is essential prior 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 β-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 physico-chemical properties and in turn has different effects on the crops' growth and yield (Table 1). Based on this it is hypothesized that vermicomposts 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 food industry sludge and cow dung-based vermicompost on growth and productivity of chickpea plants has been investigated (Table 2).

Materials and methods

The soil used in potting media was collected from the agricultural fields of Guru Jambheshwar University of Science and Technology, Hisar. The soil was sandy–loam in nature. It was deficit in nitrogen, average in phosphorus and rich in potassium content. Vermicomposts used in potting media were prepared in the laboratory using (a) cow dung and (b) wastewater treatment plant sludge of food industry spiked cow dung (Yadav and Garg 2011). The physico-chemical characteristics of soil and vermicomposts are given in Table 3. The Haryana channa-3 (HC-3) variety of chickpea was procured from the certified dealer. This variety of chickpea was chosen because of its suitability for irrigated lands, blight and wilt disease suffering areas. The main characteristics of this species are hard and bold stem and light brown grain.

The soil was fortified with 10 and 20 % (w/w, on dry weight basis) vermicompost to prepare the pot culture media. Four vermicompost-fortified potting media were prepared along with one control (soil only). The potting media were well mixed and sieved through 2 mm sieve before use. Each pot was filled with 3.0 kg of potting media and three replicates were maintained for each treatment. The composition of soil and vermicompost in different treatments is given in Table 2.

After filling potting media, the pots were maintained as such for 2 weeks at 60–70 % moisture content. After that 10 chickpea seeds were drilled in each pot at a depth of approximately 2 cm in the third week of October. The temperature is in range of 18–27 °C during this period. The chickpea seeds were treated with systemic fungicide, viz., carbendazim (50 % WP @ 1.5 g kg^{-1} seed) to protect from seedling diseases. The germination was recorded in all pots 7 days after sowing (DAS) and two healthy seedlings pot^{-1} were chosen for further study. The pots were kept in open in a pot house without controlling temperature. Plant height was recorded periodically at 30, 60 and 90 DAS by measuring the height from the ground level to the furthest extremity of the longest branch. Fresh and fully mature leaves were used to determine the chlorophyll, carotenoid and protein contents at 30 DAS. At the end of experiment, plants were removed carefully from the pots and separated in roots and shoots. Then fresh root and shoot biomass was recorded. No additional fertilizers were added during the study. The irrigation was done periodically as per requirement.

Germination percentage was calculated using the below given formula:

Table 1 Effects of different vermicompost on physico-chemical properties of soil and crop

Raw material used for vermicomposting	Ratio of mixing/dosage	Duration of study	Crop grown	Conclusion	References
Vermicompost–compost (mix) + bone meal	Vermicompost–compost mix 2 kg m^2 + bone meal, dosage 0.15 kg m^2	132 days	Beet	An increment in extractable P and stimulated microbial respiration in soil was found in all the treatments. N and P concentration in beet aerial biomass was also recorded in treatments with amendment application	González et al. (2010)
Buffalo manure	Soil + vermicompost (equivalent to 20 t ha^{-1} of organic substrate)	1 year	Maize and tomato	Vermicompost amendment modified soil chemical properties leading to higher C and N, higher pH and CEC, and lower available P, NH_4^+ and NO_3^- than in the control	Doan et al. (2013)
Buffalo manure	At the rate of 20 t ha^{-1}	3 years	Maize	CEC, N, P and K content was significantly higher at the end of the experiment in vermicompost-amended soils than control soil. The combination of vermicompost and biochar improves plant productivity and reduce the negative impact of agriculture on water quality	Doan et al. (2015)
Temple, farmyard and kitchen wastes	5, 10 and 25 % vermicompost–soil (v/v)	–	Chickpea	Vermicompost–water extract (VCE) stimulates seed germination, seedling growth and plant growth of chickpea	Singh et al. (2013)
Vegetable peels and leaf litter	10, 25 and 50 % of vermicompost–soil (v/v)	–	Chickpea	Substitution of soil with 10, 25, and 50 % VC significantly increased growth and biomass production of chickpea seedlings progressively with the increase in vermicompost substitution compared with control	Sahni et al. (2008a)
–	Vermicompost application @ 0, 1, 2 and 3 t/ha	3 years	Chickpea	The application of vermicompost improves plant growth, yield attributes and grain yield	Singh et al. (2012)
Vegetable peels and leaf litter	Garden soil substituted with different amounts of vermicompost (10, 25 and 50 % w/w)	30 days	Chickpea	Substituting the soil with different amounts of vermicompost showed significant reduction in mortality of chickpea compared to control. Maximum reduction in plant mortality was noticed in 50 % vermicompost substitution	Sahni et al. (2008b)
Paddy straw (*Oriza sativa*) and *Ageratum conyzoides* residues	1 t/ha	60 days	Zea mays, *Phaseolus vulgaris* and *Abelmoschus esculentus*	Vermicompost amendment of soil increased the nutrient supply and plant productivity	Roy et al. (2010)
Cassava peels, poultry dropping, cow dung and guava leaves	8 kg/plot (1 × 6 m)	4 months	Cowpea	This field study observed that cassava peel vermicompost enhanced cowpea aerial biomass production but acidified the soil. The effect of various vermicompost on soil physico-chemical and biological properties is very pronounced	Mba (1996)
Cattle manure, market food waste and recycled paper waste	10 or 20 t ha-1 (d.w.) to tomatoes and peppers, 5 or 10 t ha-1 (d.w.) to strawberries	For one season	Tomato, pepper and strawberry	The marketable tomato, pepper and strawberry yields in all vermicompost-treated plots were consistently greater than yields from the inorganic fertilizer-treated plots	Arancon et al. (2003)

$$\text{Percent germination} = \frac{[\text{No. of seeds germinated}]}{[\text{Total no. of seed drilled}]} \times 100$$

Chlorophyll, carotenoid and protein contents were determined at 30 DAS. Chlorophyll ‘*a*’ and ‘*b*’ contents and total chlorophyll were determined as reported by

Table 2 Composition of soil and vermicompost in different treatments

Treatment	Soil (%)	Vermicompost
T_1	100	0 %
T_2	90	10 % Vermicompost (cow dung)
T_3	80	20 % Vermicompost (cow dung)
T_4	90	10 % Vermicompost (30 % FIS + 70 % cow dung)
T_5	80	20 % Vermicompost (30 % FIS + 70 % cow dung)

Table 3 Physico-chemical characteristics of experimental soil and vermicomposts

Parameters	Soil	Vermicompost I (100 % cow dung)	Vermicompost II (70 % CD + 30 % FIS)
pH	8.5 ± 0.4	6.8 ± 0.1	6.6 ± 0.2
EC (dS/m)	0.4 ± 0.02	1.6 ± 0.04	1.8 ± 0.06
TOC (g/kg)	24 ± 1.7	280 ± 5	310 ± 8
TKN (g/kg)	1.6 ± 0.1	28.8 ± 0.35	26 ± 0.5
OM (%)	4.13 ± 0.3	48.2 ± 1.3	52.5 ± 1.15
TAP (g/kg)	0.6 ± 0.05	10.5 ± 0.4	9.75 ± 0.3
TK (g/kg)	2.0 ± 0.2	10 ± 0.2	7.6 ± 0.4
C:N ratio	15 ± 0.4	9.72 ± 0.54	11.9 ± 0.3

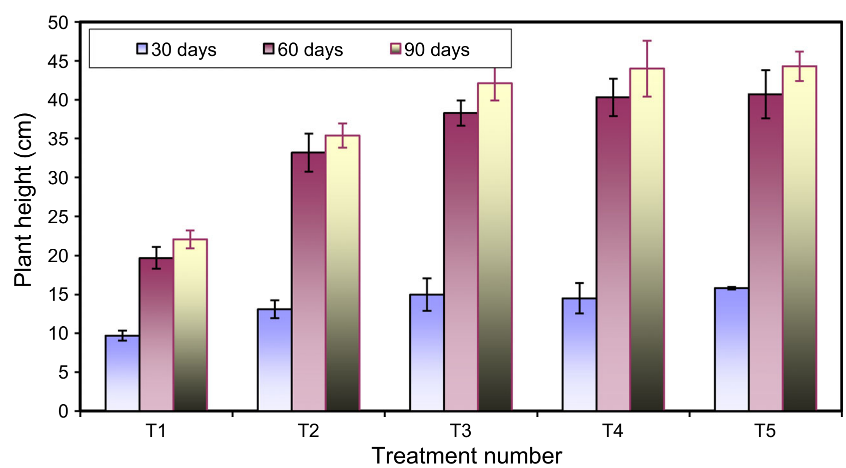

Fig. 1 Height of chickpea plants grown in different vermicompost treatments

Arnon (1949). Carotenoid was determined by the method given by Ikan (1969). Protein content in leaf tissues was estimated using the method of Bradford (1976).

The reported results are the mean of three replicates. One-way analysis of variance (ANOVA) was used to analyze the significant differences among different treatments for studied parameters. All statistical tests were evaluated at the 95 % confidence level using the SPSS software.

Results and discussion

Effects of vermicomposts on chickpea plants

Seed germination is an important and decisive phase in growth cycle of a plant since it determines plant establishment and yield. Seed germination was 96.6–100 % in different potting media. This shows that vermicomposts amendment had no antagonistic effect on chickpea seed germination. Several other authors have reported moderate or no effect of vermicompost on seed germination (Singh et al. 2013; Roy et al. 2010; Alves and Passoni 1997). In contrast, Ievinsh (2011) has reported that vermicompost addition inhibits seed germination depending on crop species and cultivar tested even at moderate concentrations.

The plant height increased progressively throughout the study period in all the plants (Fig. 1). It varied significantly in different treatments. At 30 DAS, the plant height was in the ranges of 9.7–15.8 cm in different treatments. On 30 DAS minimum plant height was in treatment T_1 and maximum was in treatment T_5. At 90 DAS plant height was

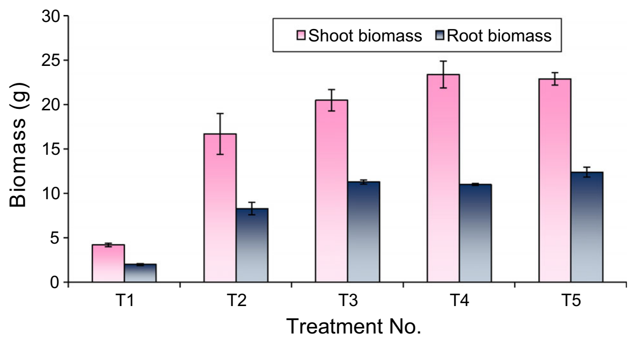

Fig. 2 Shoot biomass and root biomass in different vermicompost treatments

in the range of 22.1 (T_1) to 43.3 cm (T_5) in different treatments. Statistically, the plant height in vermicompost-fortified treatment was significantly different from control (T_1) ($F = 52.7$, $P < 0.001$). This increase in plant height may be due to enhanced supply of nutrients in the vermicompost-amended soils. Roy et al. (2010) have reported the synergistic effect of vermicompost on the plant height of maize, French bean and okra. Gopinath et al. (2008) reported that height of *Triticum aestivum* L. was significantly higher in all vermicompost and farmyard manure compost amended treatments, than the control.

Fresh shoot biomass (above ground plant weight) was measured at the end of experiment. The results of fresh shoot biomass are given in Fig. 2. Maximum shoot biomass (23.4 ± 1.7 g per plant) was recorded in treatment T_4 and minimum (4.2 ± 0.2 g per plant) in treatment T_1. The fresh shoot biomass in treatment T_3, T_4 and T_5 was not significantly different from each other. Similarly fresh root biomass was also measured at the end of experiment. Figure 2 shows the fresh root biomass recorded in different treatments. Root biomass was in the range of 2.0 (T_1) to 12.4 g per plant (T_5). The root biomass was significantly different in different treatments ($F = 98.7$, $P < 0.001$). Bachman and Metzger (2008) have also reported that the plants cultivated in media mixes containing up to 20 % pig manure vermicompost had higher shoot biomass as compared to controls.

The number of pods per plant is an important yield contributing parameter. The number of pods per plant was different in different plants grown in vermicompost spiked soil that had more pods per plant as compared to control. The number of pods per plant in different treatments varied from 5.3 to 19.3 (Fig. 3). Maximum number of pods per plant was recorded in treatment T_4 and minimum was in control. The difference in numbers of pods per plant was non-significant within different vermicompost-fortified treatments, whereas the numbers of pods per plant were significantly different in control and vermicompost-fortified treatments ($F = 12.42$, $P < 0.001$).

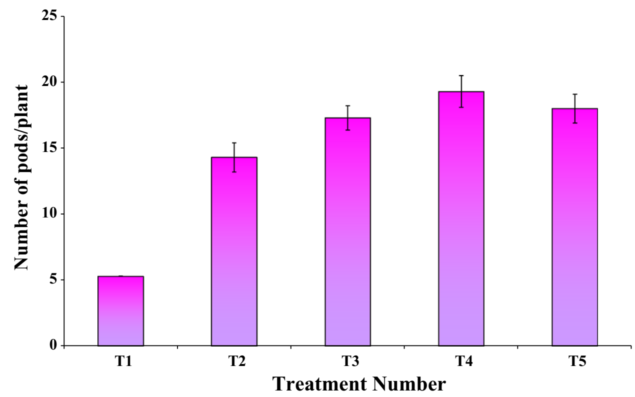

Fig. 3 Total numbers of pods in chickpea plants grown in different vermicompost treatments

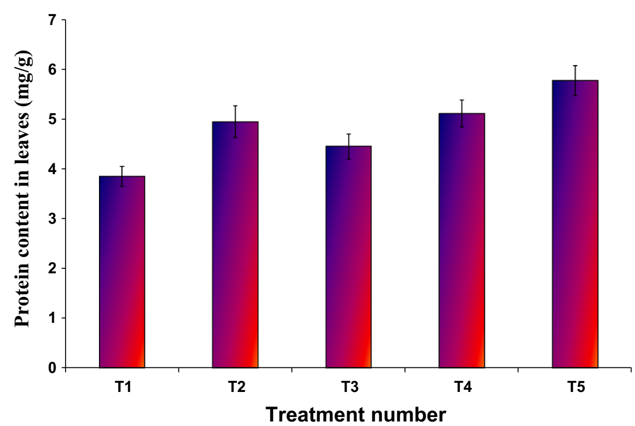

Fig. 4 Protein content in the leaves of chickpeas grown in different vermicompost treatments

Protein content in plant leaves was measured at 30 DAS and it varied from 5.83 to 3.85 mg/g in different treatments (Fig. 4). It was minimum in the leaves of chickpea plants grown in control. Maximum protein content was in the leaves of the plants grown in treatment T_5 followed by T_4. This was in agreement with reported literature that protein contents in onion grown in photo-catalytically treated dyeing industry effluent were higher than control (Ameta et al. 2003). The protein contents in treatment T_4 and T_5 were significantly different from other treatments ($F = 34.4$, $P < 0.001$).

Photosynthetic pigments

Determination of chlorophyll content in plant leaves is an indirect method of estimating the crop productivity. Chlorophyll *a*, chlorophyll *b* and total chlorophyll content measured in all the treatments on 30 DAS (Fig. 5). The chlorophyll *a* was significantly higher than chlorophyll *b* in all the treatments. Maximum chlorophyll *a* content was in

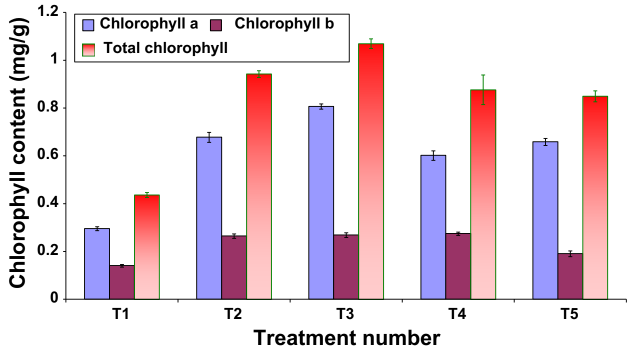

Fig. 5 Chlorophyll content in the leaves of chickpeas grown in different treatments

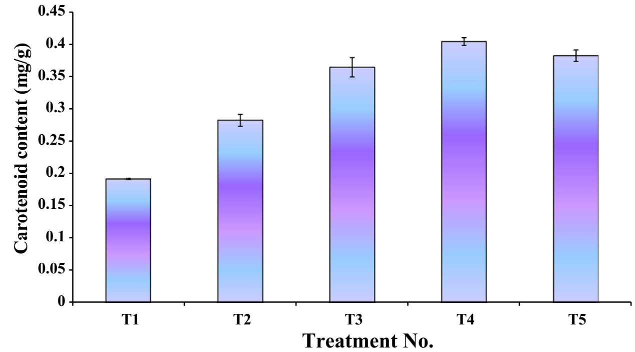

Fig. 6 Carotenoid content in the leaves of chickpeas grown in different treatments

those plants grown in treatment T_3 followed by T_5 and minimum was in control. Chlorophyll *a* content ranged from 0.296 to 0.807 mg/g in different treatments. The differences in Chlorophyll *a* content after 30 DAS between the treatments were significantly different ($F = 693$, $P < 0.001$).

Chlorophyll *b* content in plant leaves was minimum in control and maximum in treatments T_4 (Fig. 5). The chlorophyll *b* content in different treatments ranged from 0.141 to 0.275 mg/g. The difference in Chlorophyll *b* content between the treatment T_2, T_3 and T_4 was non-significant ($P = 0.65$). Total chlorophyll content ranged from 0.437 to 1.07 mg/g. Total chlorophyll content was maximum in treatment T_3 and minimum in control. The results indicated that spiking of vermicomposts with soil has a synergistic effect on chlorophyll content of chickpea leaves. The total Chlorophyll contents after 30 DAS were not significantly different from other treatments ($P = 0.99$), except control (T_1) ($P < 0.001$) Supply of more nutrients by vermicomposts as compared to soil may be the reason of enhanced leaf chlorophyll content in chickpea plants. Nitrogen is the most important mineral element in the process of chlorophyll biosynthesis, adding nitrogen to the soil may have a positive effect, which leads to increase of leaf chlorophyll content (Cecchin and Terezinha 2004). This may be the cause for higher total chlorophyll content in treatment T_3. Atiyeh et al. (2002) have reported that plants grown in 10–20 % vermicomposted food wastes contained more chlorophyll than the plants grown on control. Sangwan et al. (2010) have also reported that plants grown in cow dung vermicompost had higher total chlorophyll content than control.

Carotenoids play important role in photosynthesis process. Biosynthesis of these pigments in plants is a genetic characteristic, but some time environmental conditions also play a vital role. Figure 6 represents the carotenoid content in fresh leaf of plants measured at 30 DAS in all treatments. Carotenoid content was minimum in control and maximum was in treatments T_4. It varied from 0.191 to 0.404 mg/g in different treatments. The Carotenoid content in different treatments showed statistical significant difference ($F = 320.8$, $P < 0.001$). The more availability of nutrients in FIS containing vermicomposts may be the reason of higher carotenoid content in treatments (T_4 and T_5).

Conclusion

The results indicate that the vermicompost application has increased plant height, plant shoot biomass, seedpods, and photosynthetic pigments. Substitution of soil with 10 and 20 % vermicompost significantly increased growth in chickpea plants progressively with the increase in vermicompost substitution compared with those grown in soil (control). The increased growth may be due to enhanced capacity of plants to produce more leaves and the greater photosynthetic activity in leaves. Besides nutrient supply, the increased soil microbial activity and biomass in amended soils may indirectly have contributed to the enhanced growth and yield of plants.

Author contribution AY carried out the experiment and physico-chemical analysis and drafts the manuscript. VKG planned this study, supervision on the data analysis and revised the manuscript. Both authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest The authors declare that they have no competing interests.

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