

Utilization of Vermicompost as Growth Enhancer for Groundnut (*Arachis hypogaea* L.) under the Stress of Root Knot Nematode (*Meloidogyne arenaria*)

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Abstract

Synthetic nematicides have negative impact on soil flora and fauna hence alternative methods are needed to replace them. To materialize the thought, the current study was conducted to assess the vermicompost (VC) as an anti-nematode and plant-growth enhancer in eco-friendly manner. Seeds of groundnut (*Arachis hypogaea* L.) were sown in clay pots containing VC (10, 20, 30, 40 and 50%) amended soil. After seedling emergence plants were inoculated with *Meloidogyne arenaria*, identified by perineal pattern. Results showed that application of VC improved the soil health. It has been found that *M. arenaria* inoculation significantly reduced the growth, yield and photosynthetic pigments of groundnut plants compared to the untreated and un-inoculated plants. Plants cultivated in 40% VC (60:40 w/w soil:VC) the growth and yield substances had significantly ($P \leq 0.05$) improved as compared to untreated and nematode inoculated plants. Moreover, the photosynthetic attributes were also enhanced in 40% VC amended soil. However, VC amended soil not only increases the growth and yield but also reduces the nematode disease on groundnut plants. Therefore, our results demonstrated that VC at 40% level can be utilized to manage *M. arenaria* in eco-friendly manner through improvement in growth, yield and resistance of plants. Studied growth and yield pursuits were ameliorated gradually from untreated to 40% VC amendments. Thereby improvisation in all referred as above pursuits were looking to be VC concentration dependent.

Key words: Groundnut, *M. arenaria*, Vermicompost, Growth, Yield, Photosynthetic pigments, Sustainable approach

Northern India is one of the major agriculture producers, facing many problems including environmental changes. Among them, uneven rainfall causing floods or drought in alternate years and subsequent reduction impacts go on to the growth and yield of major rain fed growing crops. So, it looks the need of the hour to devise high value and short duration crops which could cope up in such unpredictable climatic conditions and prevents large yield losses.

Groundnut (*Arachis hypogaea* L.), is a valuable and major oil-seed crop belonging to the family Fabaceae. It is cultivated as a different adaptation strategy for poor farmers who entirely depend on farming [1-2]. Approximately 75% of India's groundnut-producing land is located in a zone with low to moderate rainfall and a brief growth season (90 to 120 days). About 80% of groundnut crops are produced during the kharif season, from June to September. However, groundnut production varies with monsoon in several areas. It is grown during the pre- and post-monsoon seasons in south and eastern areas of country. Moreover, it can also be grown as a summer crop from January to May if irrigation facilities are adequate. Bandyopadhyay *et al.* [3] observed that groundnut cultivation under proper irrigation throughout the summer months (March–June) may enhance the productivity by two to three times

relative to the monsoon crop. Apart from these, phyto-nematodes are the major hurdles to groundnut production globally. Several phyto-nematodes including root-knot nematodes (RKNs) (*Meloidogyne* spp.) significantly lower the groundnut production every year [4-5]. Among RKNs, *M. arenaria* is most prevalent species causing significant yield loss to groundnut crop by forming galls on the root [6]. However, yield loss also depends upon climatic conditions, nematode population and plant cultivar. To manage nematodes, various approaches have been developed worldwide. One of them, synthetic nematicides are quite successful, but their regular usage proved to be harmful for both environment and human [7]. Hence, there is need to develop alternatives for managing RKNs.

Vermicompost (VC) is the example of organic matter utilized for the management of *M. arenaria* in eco-friendly manner. It converts organic waste into high-valuable products in the soil and utilized widely in less industrialized countries during the last 40 years [8]. It has also been proven more efficient both as an organic fertilizer and bio control agent with than its counter parts due to the addition of thermophilic aerobic compost [9-11]. From the last decade, use of liquefied VC such as extracts of teas increase, in addition to use solid VC for plant

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disease management [12-14]. Both forms of VC (solid and liquid) have a high potential for crop protection against numerous pests, and have possibility in sustainable organic cropping systems. Apart from this, VC is rich source of inorganic nutrients such as nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), manganese (Mn), zinc (Zn) and copper (Cu) which enrich the soil with different micro and macronutrients [15-16]. It is also thought to be an effective organic matter supplement to the soil that increasing soil fertility. Recently, VC has also been thoroughly investigated for its many other beneficial properties, namely its ability to promote plant growth and inhibit plant diseases through a variety of pathways [17]. Because of its superior ability to alter soil qualities, it is regarded as preferable to traditional compost, particularly when used as a substitute for soils or greenhouse container media. The role of VC is generally assumed to be owing to its nutrient-rich composition as well as its ability to affect soil physical and biochemical properties in a way that favors plant growth and development [8]. In addition to suppressing phytopathogens and nematodes, it is thought to alter a plant's internal defense response to microbial attack. Rehman et al. [18] found that VC-mediated soil promotes the multiplication of saprophytic soil microorganisms, including bio-control agents, and hence improve the effectiveness of several bio-control agents against a wide range of phytopathogens, particularly RKNs. VC also increase oxygen availability of soil, maintain soil temperature, increase soil porosity and nutrient content [19].

However, there is meager information with regards to VC impacts on plant growth particularly in those plants growing under the stress of RKNs. So, this part of research work was modulated to evaluate the groundnut growth and yield in VC (at different levels) amended soil with RKNs.

MATERIALS AND METHODS

Identification of collected RKN species

Galled root samples were obtained from groundnut fields and carry for identification of nematode species in the

laboratory. Perineal pattern method was followed for the identification of species [20]. According to this method, about ten RKN females were taken from the galls, and slides for pattern were prepared under microscope (dissecting). Furthermore, the posterior portion of the female was cut using sharp blade and needle and the pattern was trimmed into square shape and examined under light microscope [21]. The nematode species was identified as *M. arenaria* (Fig 1), based on characteristics described by Eisenback *et al.* [20].

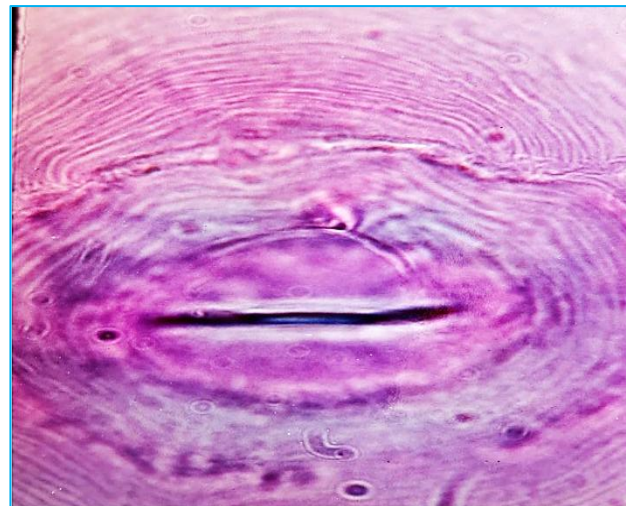


Fig 1 Microscopic view of perineal pattern of *M. arenaria*

Preparation and maintenance of *M. arenaria* inoculum

Pure population of *M. arenaria* was maintained on brinjal plants in pots for further experiment. Egg masses of nematodes were handpicked from the roots of brinjal plants and kept in petri plates with distilled water (DW) to favor the egg hatching at 27 ± 2 °C. After hatching, juveniles at second stage (J2s) were obtained from suspension, and DW was added to petri plates. Freshly hatched J2s were used as inoculum for pot study while remaining J2s maintained and store at 4-10 °C for further use (Fig 2). *RKN*

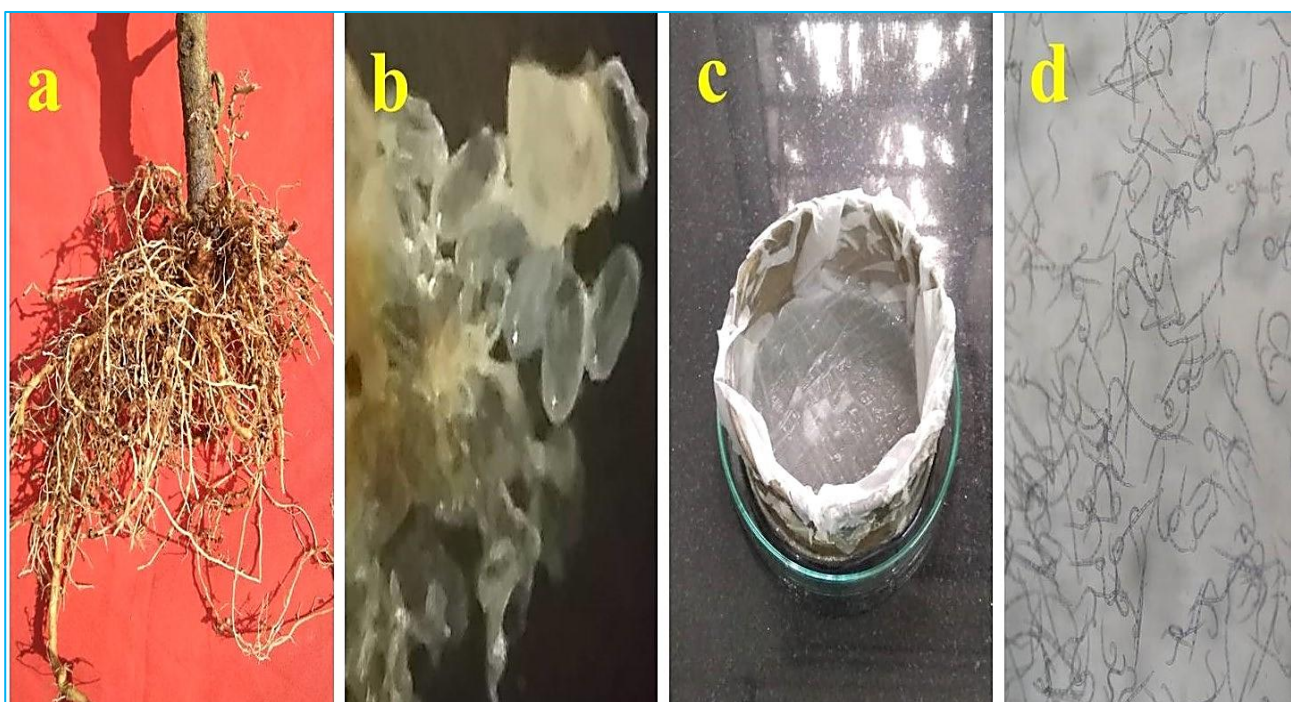


Fig 2 Figure shows the preparation of *M. arenaria* inoculum, (a) infected eggplant root, (b) egg mass, (c) Sieve with eggs in petri plate, (d) freshly hatched second stage juveniles

Collection of vermicompost (VC) and soil

For the experimental work, VC was collected from agriculture seed shop, located at near railway station, Aligarh, UP, India (Fig 3). While soil was collected from agriculture field up to 20 cm of depth, after removing the unnecessary litters and soil particles.



Fig 3 Figure shows the vermicompost as plant growth enhancer and anti-nematode material against *M. arenaria*

Experimental performance and design

All the pot experiments were performed in clay pots (3 kg soil/pot) in Net House, D. S. College, Aligarh, UP, and designed in a randomized complete block. Collected soil was sterilized in autoclaved at 15 lb. pressure for 20 minutes before experiment. After sterilizing the soil, experiment was categorized into seven groups with five replicates for each treatment. The first group: untreated and un-inoculated control (UUC) means the plants not treated with VC and not inoculated with *M. arenaria*. Second group: untreated and inoculated control (UIC) means plants not treated with VC while inoculated with *Meloidogyne arenaria* J2s. Third group: 10% VC + *Meloidogyne arenaria*. Fourth group: 20% VC + *Meloidogyne arenaria*. Fifth group: 30% VC + *Meloidogyne arenaria*. Sixth group: 40% VC + *Meloidogyne arenaria*; seventh group: 50% VC + *Meloidogyne arenaria*. All the seeds were surface sterilized in 1% NaOCl solution before sowing to the pots (5 in each). After seedling emergence, thinning was done in all pots to maintain a healthy seedling. Then, inoculation was done with fresh hatched J2s of *Meloidogyne arenaria* at 2000 J2s/pot on the basis of experimental design. Every pot containing groundnut seedlings in holes received 5 ml of nematode suspension. The majority of the J2s swiftly approached the roots since 3 to 5 holes were carefully drilled without causing any harm to the root. To prevent dry conditions, all the plants received frequent irrigation.

Observations

Plants were uprooted after 3 months of J2s inoculation and washed with water to remove the soils. Data were collected as plant growth, yield, photosynthetic pigments like chlorophyll a, b and carotenoids and disease parameters viz., root-knot index (RKI), egg masses/root system and nematode population (NP).

Growth and yield attributes

Growth attributes such as shoot and root length, fresh and dry weight (shoot and root), while yield such as number and area of leaves, number of pods and flowers were measured. Weight as fresh and dry were measured using weighing balance than followed by oven drying at 80 °C for 2-3 days.

Photosynthetic attributes

Chlorophyll and carotenoid estimation

Fresh groundnut plant leaves were examined for photosynthetic pigments such as chlorophyll (a and b) and carotenoids using the Mackinney [22] method. According to this method, fresh leaf of groundnut (1g) was crushed into a powder form and mixed with 20 ml acetone (80%). For chlorophyll estimation absorbance was measured at 645 and 663 nm wavelength and carotenoid at 480 and 510 nm against 80% acetone as blank using spectrophotometer.

Formulae were given below for the estimation of chlorophyll and carotenoids:

$$\text{Chl. 'a'} = 12.7 (A_{663}) - 2.69 (A_{645}) \times (V/(W \times 1000))$$

$$\text{Chl. 'b'} = 22.9 (A_{645}) - 4.68 (A_{663}) \times (V/(W \times 1000))$$

$$\text{Carotenoids} = 7.6 (A_{480}) - 1.49 (A_{510}) \times (V/(W \times D \times 1000))$$

Where,

A = Absorbance, W = Weight of leaf sample, V = final volume taken, D = Path length of light

Disease attributes

Estimation of egg masses number

Egg mass number in infected roots of groundnut plants was measured according to method proposed by Holbrook et al. [23]. According to this method, roots were washed with water and stained with Phloxine B (0.15 g/L water) for 5 – 10 minutes, extra stain washed and egg masses number were count manually.

Estimation of nematodes number

Cobb's [24] sieving and decanting method, followed by Baermann's funnel [25] technique, was used to determine the nematode number from 250 g of soil per treatment. Five replicates per treatment were employed, and aliquots of 1 ml of the prepared culture were sampled under a stereomicroscope to determine the number of nematodes after 72 hours.

Root-knot index

Taylor and Sasser [26] method was used for the measurement of RKI by applying the 0-5 scale i.e., 0 = No galls, 1 = 1–2 galls, 2 = 3–10 galls, 3 = 11–30 galls, 4 = 31–100 galls and 5 = more than 100 galls per root system.

Data analysis

The data for pot experiments are the average of five replicas. Data was measure by analysis of variance (ANOVA) using SPSS-17.0 statistical software (SPSS Inc., Chicago, IL, USA) to determine the significant at $P \leq 0.05$. Significant differences between treatments were analyzed by Duncan's multiple range test (DMRT).

RESULTS AND DISCUSSION

Effects of VC on growth and yield of groundnut crop

Results shown in (Table 1, Graph 1) revealed that growth and yield (shoot and root length, shoot and root fresh and dry weight, leaf area, number of leaves and pods) of groundnut plants under the influence of *M. arenaria* are increased by the soil application of VC from 10 to 50%, as compared to untreated and nematodes inoculated control (UIC). The results showed at 40% of VC, the significant increase in growth and yield of groundnut plants in terms of shoot and root length (46.81 and 24.27 cm), shoot and root fresh weight (43.73 and 10.19 g), shoot and root dry weight (10.01 and 3.35 g), number of leaves (81.75), leaf area (11.71 cm²/plant), number of flowers and pods (13.31 and 14.68) of groundnut crop, as compared to UIC (Fig 4).

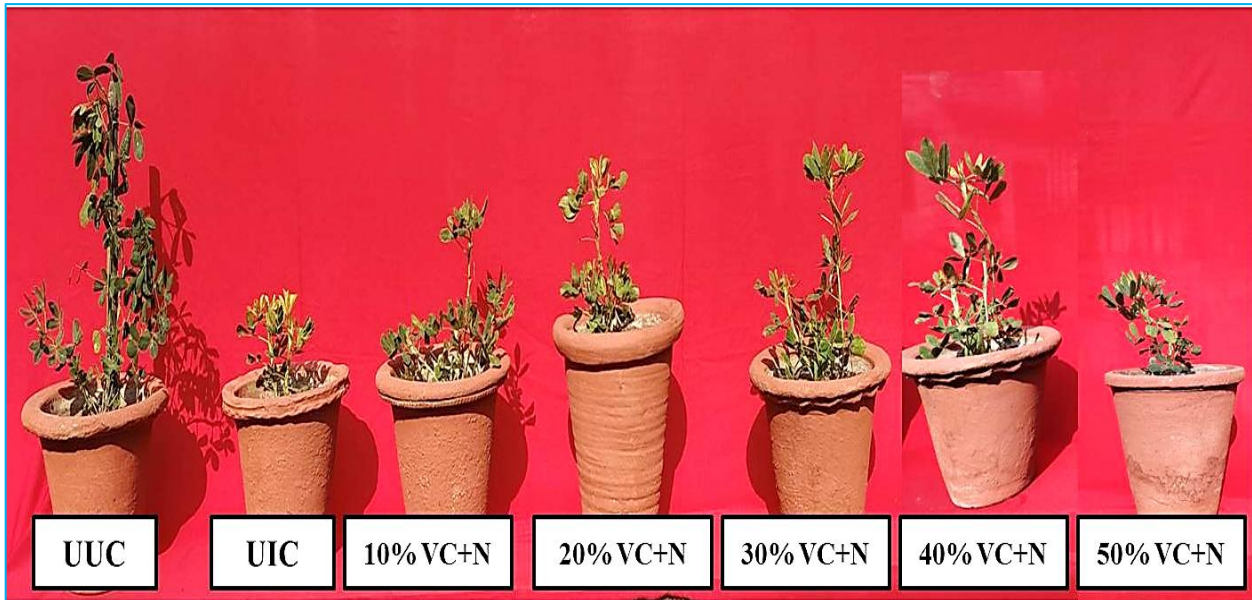


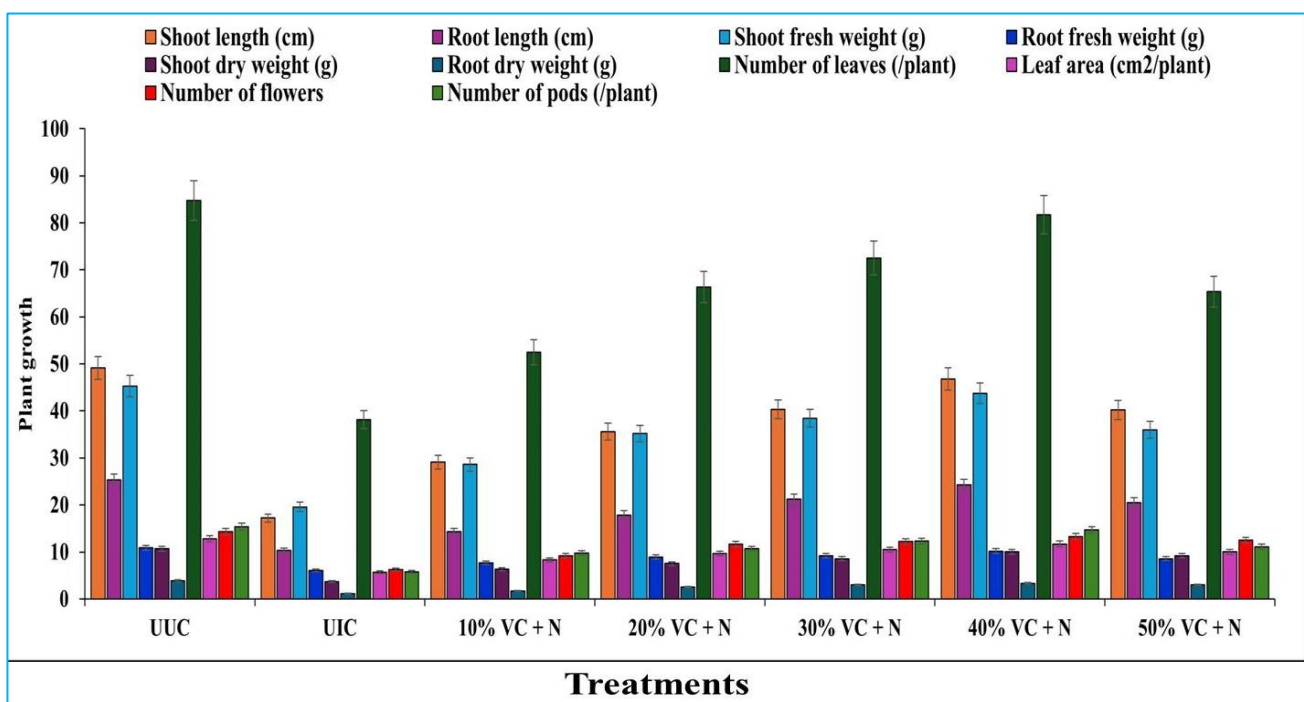
Fig 4 Figure shows the interactive effect of vermicompost (10-50%) and *M. arenaria* on the growth and yield of groundnut plants
 UUC = Untreated uninoculated control; UIC=Untreated inoculated control with *M. arenaria*; VC = vermicompost; N = Nematodes

Table 1 Effect of different levels of vermicompost on *Meloidogyne arenaria* in relation to plant growth and yield attributes of groundnut crop

Attributes	Treatments						
	UUC	UIC	10% VC + N	20% VC + N	30% VC + N	40% VC + N	50% VC + N
Shoot length (cm)	49.12 ^a	17.22 ^f	29.11 ^c	35.61 ^c	40.35 ^b	46.81 ^c	40.21 ^d
Root length (cm)	25.35 ^a	10.31 ^e	14.33 ^d	17.87 ^c	21.21 ^b	24.27 ^c	20.51 ^d
Shoot fresh weight (g)	45.25 ^a	19.59 ^f	28.61 ^c	35.17 ^c	38.44 ^b	43.73 ^c	36.01 ^d
Root fresh weight (g)	10.88 ^a	6.05 ^f	7.65 ^e	8.91 ^c	9.23 ^b	10.19 ^c	8.55 ^d
Shoot dry weight (g)	10.67 ^a	3.72 ^f	6.31 ^e	7.54 ^c	8.55 ^b	10.01 ^c	9.21 ^d
Root dry weight (g)	3.88 ^a	1.15 ^e	1.73 ^d	2.55 ^c	3.01 ^a	3.35 ^b	3.00 ^d
Number of leaves (/plant)	84.71 ^a	38.19 ^g	52.49 ^f	66.33 ^d	72.54 ^b	81.75 ^c	65.37 ^e
Leaf area (cm ² /plant)	12.81 ^a	5.69 ^f	8.31 ^e	9.65 ^d	10.49 ^b	11.71 ^c	10.02 ^d
Number of flowers	14.31 ^a	6.25 ^g	9.21 ^f	11.62 ^d	12.23 ^b	13.31 ^c	12.49 ^e
Number of pods (/plant)	15.37 ^a	5.79 ^f	9.78 ^e	10.69 ^d	12.31 ^b	14.68 ^c	11.11 ^d

Each value is the mean of five replicates

Values in each column followed by same letter are not significantly different according to Duncan's Multiple Range Test (DMRT) at P ≤ 0.05
 UUC – Untreated uninoculated control; UIC – Untreated inoculated control; VC – Vermicompost, N – Nematodes (*M. arenaria*)



Graph 1 Effect of different levels of vermicompost on *M. arenaria* in relation to plant growth and yield attributes of groundnut crop

Effects of VC on photosynthetic pigments of groundnut crop

Results shown in (Table 2, Graph 2) revealed that all the photosynthetic pigments like chlorophyll 'a', 'b' and carotenoids of groundnut plants under the influence of *Meloidogyne arenaria* are increased by the soil amendment

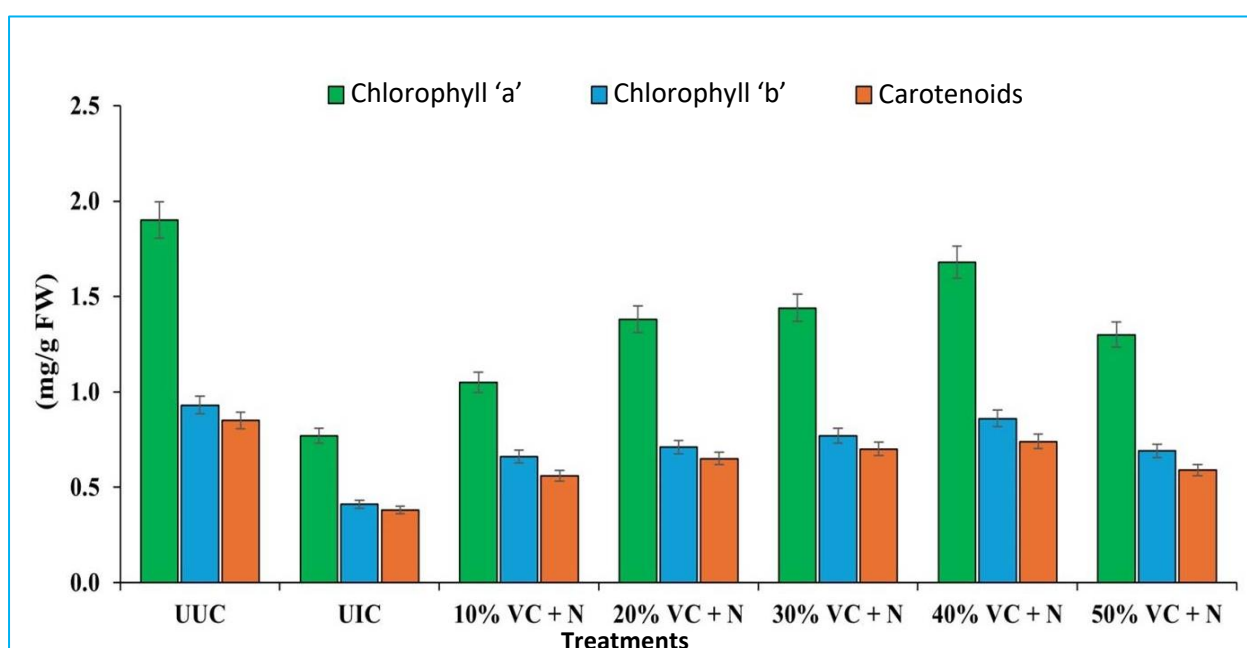
with VC from 10 to 50%, as compared to UIC. The results revealed at 40% of VC, the significant increase in photosynthetic attributes like chl. a (1.68 mg/g), chl. b (0.86 mg/g) and carotenoids (0.74 mg/g) of groundnut crop, as compared to untreated inoculated control (UIC).

Table 2 Effect of different levels of vermicompost on *M. arenaria* in relation to photosynthetic attributes of groundnut crop

Treatments	Photosynthetic attributes		
	Chlorophyll 'a' (mg/g Fresh weight)	Chlorophyll 'b' (mg/g Fresh weight)	Carotenoids (mg/g Fresh weight)
Untreated uninoculated control	1.90 ^a	0.93 ^a	0.85 ^a
Untreated inoculated control	0.77 ^f	0.41 ^e	0.38 ^f
10% Vermicompost (VC) + Nematodes (N) (<i>M. arenaria</i>)	1.05 ^e	0.66 ^d	0.56 ^e
20% Vermicompost (VC) + Nematodes (N) (<i>M. arenaria</i>)	1.38 ^e	0.71 ^c	0.65 ^c
30% Vermicompost (VC) + Nematodes (N) (<i>M. arenaria</i>)	1.44 ^b	0.77 ^b	0.70 ^b
40% Vermicompost (VC) + Nematodes (N) (<i>M. arenaria</i>)	1.68 ^c	0.86 ^c	0.74 ^c
50% Vermicompost (VC) + Nematodes (N) (<i>M. arenaria</i>)	1.30 ^d	0.69 ^d	0.59 ^d

Each value is the mean of five replicates

Values in each column followed by same letter are not significantly different according to Duncan's Multiple Range Test (DMRT) at P ≤ 0.05



Graph 2 Effect of different levels of vermicompost on *M. arenaria* in relation to photosynthetic attributes of groundnut crop

Table 3 Effect of different levels of vermicompost on root-knot disease caused by *M. arenaria* in groundnut crop

Treatments	Pathological attributes		
	Number of egg masses/root system	Nematode population/250g of soil	Root-knot index (RKI)
Untreated uninoculated control	0.00	0.00	0.00
Untreated inoculated control	110.34 ^a	1915.22 ^a	5.78 ^a
10% Vermicompost (VC) + Nematodes (N) (<i>M. arenaria</i>)	68.43 ^b	1108.31 ^b	2.19 ^b
20% Vermicompost (VC) + Nematodes (N) (<i>M. arenaria</i>)	25.11 ^c	830.22 ^c	1.75 ^c
30% Vermicompost (VC) + Nematodes (N) (<i>M. arenaria</i>)	1.05 ^d	100.01 ^d	1.00 ^d
40% Vermicompost (VC) + Nematodes (N) (<i>M. arenaria</i>)	0.00	0.00	0.00
50% Vermicompost (VC) + Nematodes (N) (<i>M. arenaria</i>)	0.00	0.00	0.00

Each value is the mean of five replicates

Values in each column followed by same letter are not significantly different according to Duncan's Multiple Range Test (DMRT) at P ≤ 0.05

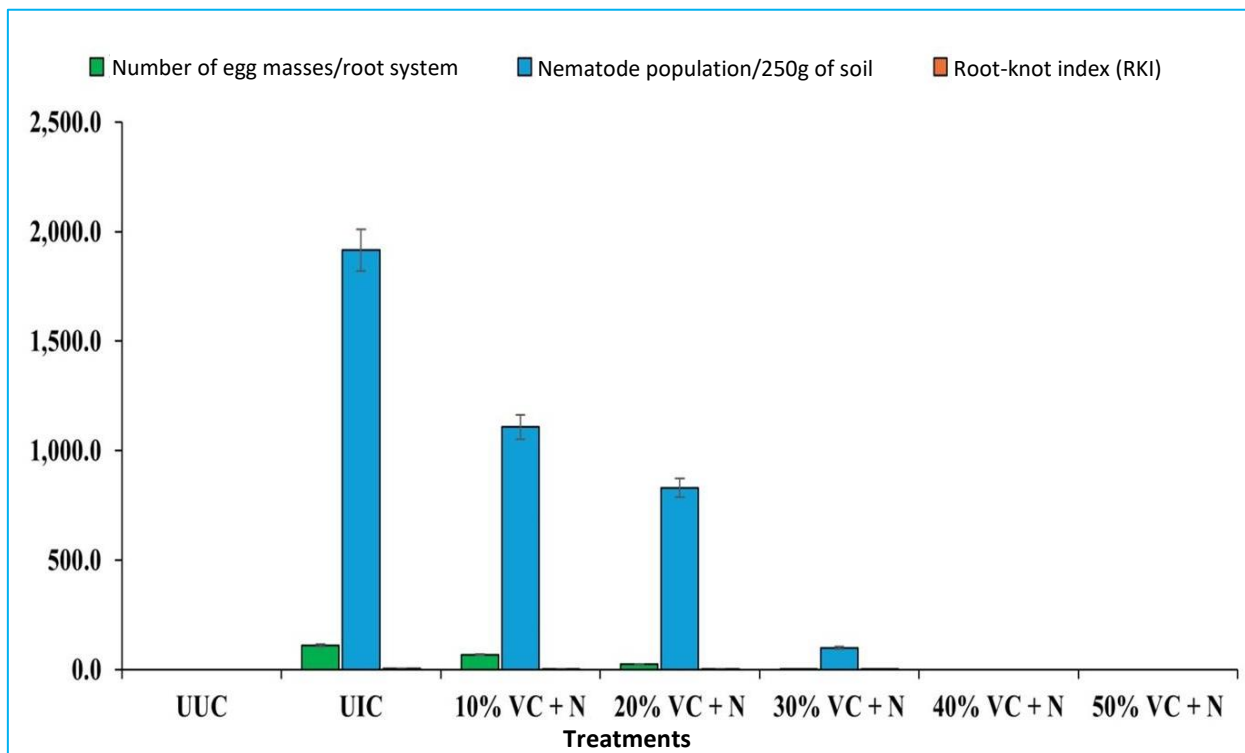
Effects of VC on disease performance of M. arenaria

The results shown in (Table 3, Graph 3) revealed that the untreated and nematode inoculated plants had the highest disease in terms of number of egg mass, nematode population and root-knot index. However, the vermicompost levels from

10 to 50% significantly decreased the disease infestation (number of egg masses, nematode population and RKI) compared to UIC. Moreover, the minimum reduction in disease was found at 10% of vermicompost treated plants while complete suppression of disease was found at 40% and 50% of

vermicompost as compared to UIC. Our results showed that the disease on groundnut plants significantly decreased with increasing vermicompost concentrations. Vermicomposting is a method in which earthworms change organic waste into secondary product called vermicompost (Fig 3), which can be utilized as organic fertilizer for in agriculture [27]. Therefore,

vermicomposting is an interesting approach for recycling the organic waste into usable form. Also lower the use of fertilizers and synthetic pesticides for managing soil-borne pathogens as well. Furthermore, wide-scale application of compost is a good approach to boost the soil's organic content, which is crucial for their long-term fertility [28].



Graph 3 Effect of different levels of vermicompost on root-knot disease caused by *M. arenaria* in groundnut crop

VC is an organic fertilizer with high amount of humus, inorganic nutrients viz., N (2 - 3%), P (1.55 - 2.25%), K (1.85-2.25%), mycorrhizal fungi and nitrogen-fixing bacteria. Soil application of VC increases the actinomycetes and N-fixing bacteria in the soil. Scientifically VC has been proved as wonder plant growth stimulant [29]. Ansari and Ismail [30] also revealed that VC contains N (7.37%) and P as P_2O_5 (19.58%). Apart from this, vermicomposting is a biological, aerobic process that effectively transforms environment friendly humus-like materials [31]. Additionally, VC may have antinematode action because of its abundance in humic acid and hormones including indole acetic acid (IAA), gibberellins and cytokinins that inhibit nematode infestation [32-33].

Pot studies revealed that the growth and yield of groundnut were increased gradually up to 40% level of VC. Maximum improvement in growth and yield of groundnut crop was found in the soil amended with 40% VC, because of large amount of humus, N, P and K in the soil that make root to absorb nutrients from the soil. According to Atiyeh et al. [34], when all necessary nutrients were provided, the growth of marigold and tomato seedlings in a common horticultural, greenhouse container bedding plant medium Metro-Mix 360 (MM360) was much improved by substituting pig solids VC or food wastes VC. Raspberry plants cultivated in mineral soil containing pig manure (VC) had a higher shoot dry weight than plants growing in unfertilized control soil, and their growth was similar to that of soils treated with full fertilizer. According to Baskaran et al. [35], addition of VC to soil contaminated by sugar mill effluent increased soil fertility and promoted the growth of green gram plants. Karmegam and Daniel [36] also showed that VC had a comparable impact on hyacinth bean's growth and production. Warman and Anglopez [37]

demonstrated that, despite the VC extract's negative effect on seed germination of marigold, radish and upland cress, adding 10% VC generated from various feedstock enhanced the leaf area and biomass of all these three plant species. Sallaku et al. [38] demonstrated that VC has a good impact on plant growth even in saline environments. When compared to commercial peat compost, immature cucumber seedlings grown in VC showed a noticeably higher relative growth rate during the nursery stage, which persisted until the stand establishment phase. There were morphological variations at that point, but there were even more notable disparities in the transplanted seedlings' physiological performance. However, vermicompost had higher relative leaf expansion rate and dry matter per plant before transplanting and at the conclusion of the stand establishment period.

Results given in (Table 1) showed that 40% vermicompost level was found to maximize the growth, and yield of groundnut plants inoculated with *M. arenaria*. It appears that groundnut plants utilize the nutrient from 40% vermicompost enriched soil most appropriately compared to other levels of vermicompost even in presence of nematodes. This approaches of discussion evident from the higher ameliorated growth and yield of groundnut in such combination. Similar results were also proposed by Kumari and Ushakumari [39] on growth and yield of cowpea under the field conditions amended with vermicompost. Chamani et al. [40] also correlated the beneficial implications of VC and its impacts on growth of *Petunia hybrida* compared to control and peat-amended media. All the physiological, anatomical and metabolic transformations might have occurred properly due to the availability of different micro and macronutrients in vermicompost amended soil.

Lowering in photosynthetic rate at high levels of VC was related to the reduction in chlorophyll content of leaves. Data given in (Table 2) revealed that there is significant enhancement in photosynthetic attributes like chlorophyll a, b and carotenoids in 10% and onward level of VC but maximum being at 40% level in presence of *M. arenaria*. However, all kind of evaluated pigments were found in somewhat reduced quantities if compared to UUC treatments, however reverse were true with UIC treatments. It is thereby showing that optimal level of VC to maximize the pigment content of groundnut leaves is 40% because in onward amendments there amounts reduced. Recently, Hosseinzadeh *et al.* [41] also observed that the VC influenced the photosynthetic content of chickpea and there was gradual increase in their value from 10% to 30%, being maximum at 30% VC. Similar conclusions were drawn by Lim *et al.* [42] with regard to pigment content at 40% VC amended soil. Lim *et al.* [42] also found that in higher amounts, the harmful effect of VC is due to the high concentrations of soluble salts available in VC.

Findings of the pots' study also revealed that different VC levels (10-50%) exhibit the nematocidal activity against the survival of *M. arenaria* in terms of several pathological parameters (Table 3). VC amendment to soil from 10 to 50% reduced the number of egg masses/root system, nematode population/250 g of soil and root-knot index (RKI) caused by *M. arenaria*. Furthermore, complete reduction of nematode disease was found at 40% and 50% VC level. Our results confirmed to those of Xiao *et al.* [43], who suggested that VC significantly decreased the numbers of nematode-induced galls on tomato plants. Villenave *et al.* [44] also found that VC modifies the population of nematodes in the soil. Previous studies also suggested that humic acids and hormones viz., IAA,

cytokinins and gibberellins in VC could suppress nematode infestation [32-33].

CONCLUSION

Organic amendment with VC could help the groundnut plants resist against RKN by activating the root defense and modify the soil physio-chemical composition. In addition to improving plant resistance and soil characteristics against RKN, VC also considerably enhanced soil quality, including pH and microbial activity, which may together promote plant growth and tolerance to RKN infections. The current study examined the overall performance of groundnut plants under the influence of *M. arenaria* by demonstrating the impact of varying VC levels on agricultural soil. The 40% level was the most optimal in all the VC levels (10–50%). Results showed that at 40% VC level, growth, yield and leaf pigments of groundnut plants were significantly improved. Moreover, the significant reduction in root-knot disease in terms of pathological parameters was seen when VC levels raised from 10 to 50% on the groundnut plants. Therefore, using VC as a nematicide-cum-fertilizer is an economical way to ensure that VC is used sustainably in agro-ecosystems. Finally, the study concluded that the optimal level of VC in the soil for both managing *M. arenaria* and promoting groundnut plant development and yield is 40%.

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