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Effects of N fertilisation, organic matter, and biofertilisers on the growth and yield of chilli in relation to management of plant-parasitic nematodes

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Abstract: An experiment was conducted at the University Agricultural Research Farm to study the effect of inoculation with biological nitrogen fixers on growth and yield of chilli (*Capsicum annum* L.) cv. "Pusa Jawala" in relation to disease incidence caused by plant-parasitic nematodes. The growth, yield, and quality parameters of chilli increased significantly with the inoculation of biological nitrogen fixers using *Azospirillum* and *Azotobacter*. Performance of *Azospirillum* was found better as compared to *Azotobacter.* Simultaneous inoculation with biofertilisers (100% recommended dose of N-fertiliser 100 kg N per ha and farmyard manure 15 t per ha) resulted the maximum growth, yield, and quality parameters. Thus, the associative nature of the above biofertilisers helps to save 25% nitrogenous fertiliser in chilli crop. There was increased content in plant nitrogen, phosphate and potash, leaf chlorophyll and residual available soil nitrogen, phosphate and potash with dual inoculation with the biological nitrogen fixers along with recommended full dose of nitrogen fertiliser. Disease intensity was recorded in decreasing order in all the treatments but more pronounced in those where biofertilisers were added.

Key words: *Azospirillum*, *Azotobacter*, organic matter, nitrogen fertiliser, plant-parasitic nematodes, chilli

Introduction

Chilli (*Capsicum annuum* L.) is one of the important vegetable-cum-spice crops of India. It has different types of protein, vitamin, and ascorbic acid contents, and is a good source of medicinal potential. This crop is very important for agricultural economy and is used in processing industries. According to the Food and Agriculture Organization (FAO) of the United Nations (FAO, 2003), globally the percentage change in the area and production of chilli are consistently increasing but the productivity of chilli in India is 9.18 t/ha whereas it is 14.4 t/ha of the world. The reduction trend in the last few decades seems to be due to some pathogenic agencies as well as seasonal fluctuations. Plant pathogens decreased production across the world annually by more than 20% on average; however, individual fields may sustain losses of 50%-100% from one or more pests (Dhaliwal & Koul, 2007). Plant-parasitic nematodes are one of the major biological constraints around the world in almost all types of crop plants and they cause severe losses in the productivity of chilli (Kumar et al., 1992; Tiyagi et al., 2009; Moon et al., 2010). Chemical pesticides, due to their high availability and easy applicability, are generally preferred for their control; however, their excessive

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and continuous use was reported to have resulted in the development of pesticide resistance (Schmutterer, 1981), caused direct toxicity to predators, pollinators, fish, and man (Pimentel, 1981), had adverse effects on soil health and the environment (Diwedi & Diwedi, 2007), and caused poor soil health, fertility, and productivity (Singh et al., 2007). Any alteration in the ecological set up of biological components leads to environmental degradation. Deregistration of many pesticides and contamination of the biosphere have emphasised the need for new methods to control nematodes and subsequently improving crop productivity. Organic sources of nutrients like farmyard manures, composts, and botanical residues are extensively used in various crops. Under integrated nutrient management programmes, the best available alternative lies in the complimentary use of biofertilisers and organic manures in suitable combinations with chemical fertilisers. This ensures higher productivity, minimises expenditure on costly fertiliser inputs, improves the efficiency of added fertilisers, and at the same time yields good soil health (Bandyopadhyay & Puste, 2002; Luxminarayana & Patiram, 2006). Biological nitrogen fixers help to enhance productivity by biological nitrogen fixation, producing hormones, vitamins, and other growth factors required for plant growth and development, solubilisation of phosphorus, and suppression of growth of plant pathogens (Windhum et al., 1989; Devidas & Rehberger, 1992; Verma et al., 2000) on several crops. However, the information on integrated nutrient and pest management of chilli is meagre. Free living nitrogen fixers belonging to *Azotobacter* and *Azospirillum* live in association with the root system of crop plants and harvest atmospheric nitrogen, which in due course is made available to the plant or released in the soil. These biofertilisers are ecologically sound, economically viable, and partial substitutes for costly and pollution-causing nitrogenous chemical fertilisers. Keeping in view the importance of chilli, its lower production rate seems to be due to soil pathogenic nematodes. It is therefore worthwhile to conduct a preliminary soil-survey for the presence of plant-parasitic nematodes. The survey was conducted in infected fields of chilli plant in Aligarh and nearby districts and their presence with poor and unthrifty crop growth was observed. The affected plants showed stunted growth and developed root galls, caused by *Meloidogyne incognita*. This nematode causes knots in the roots of affected plants. Other nematode species also damage the plants and their presence is confirmed by the presence of nematodes after extraction using different sieves of various mesh size. The aim of the present study was to evaluate the effect of different nitrogen levels, organic matter in the form of neem cake, and N-fixing biofertilisers, such as *Azotobacter* and *Azospirillum*, on several growth parameters, such as plant height, number of branches/plant, percent pollen fertility, green fruits/plant, green fruits yield/plant, 100 green fruits/plant weight, chlorophyll content, ascorbic acid content, soil fertility, and subsequently on the population of plant-parasitic nematodes.

Materials and methods

A field experiment was conducted during 3 winter seasons of 2006-2009 at the University Agricultural Research Farm, Aligarh Muslim University, Uttar Pradesh, to assess the effect of inoculation of biological nitrogen fixers, such as *Azotobacter chroococcum* and *Azospirillum brasilense*, singly or in combination along with neem cake and recommended levels of inorganic fertilisers on growth, yield, and quality parameters of chilli (*Capsicum annum* L.) variety "Pusa Jawala". The experimental field was protected with barbed wire and thoroughly ploughed. The small beds of 6 m^2 were prepared leaving 0.5 m buffer zone between them. The texture of the soil was sandy loam, having pH of 6.2, 6.5, and 6.9, organic carbon 1.03%, 1.09%, and 1.16%, and available N, P, and K of 138.30, 162.56, and 186.39; 41.34, 43.66, and 46.66; and 55.61, 62.13, and 66.45 kg/ha, respectively, for the respective years. These beds were separately treated with neem cake 110 kg N/ha, and immediately watered to assist in the decomposition of cake. Before the treatment, the initial nematode population from the individual beds was taken. After 15 days of seed treatment with *Azotobacter* and *Azospirillum* each at 25 g/kg seed for 20 min, seedling root dip at 2% for 30 min at the transplanting stage (Kavitha et al., 2003) as single and in dual inoculation was tested with 3 levels of N (50%, 75%, and 100%) of the recommended dose. Untreated beds and those treated with 100% recommended N P K served as control. The treatments were randomised with 5 replications.

The planting of chilli seedling at 4 weeks of age was done at 30 cm space from each other in each bed. Nitrogen was applied in the form of urea depending upon the treatments in split doses, half of the basal and another half was applied in 2 equal splits at 3 and 6 weeks after transplanting. Phosphate and potash (each 60 kg/ha) were applied in the form of single super phosphate as the basal and muriate of potash in 2 equal splits, and as the basal and the rest at 6 weeks after transplanting to all the beds. Necessary watering and weeding were performed as and when required. Various growth, yield, and quality parameters were measured separately over 3 years.

When the plants reached the subsequent stage, the pollen fertility was estimated by the method of Brown (1949) using acetocarmine for staining the pollen grains. The ascorbic acid content of chilli fruit tissue was determined by the method based on the reduction of 2, 6-dichlorophenol indophenol dye (Roe, 1954). Chlorophyll content of leaf was estimated by the method of Hiscox and Israelstam (1979). One hundred milligrams of leaf pieces was placed in a vial containing 7 mL of dimethyl sulfoxide (DMSO) and the chlorophyll was extracted into the fluid by incubating for 60 min. The extracts was transferred to a graduated tube and made up to 10 mL with DMSO and assayed immediately. A sample of 3 mL of chlorophyll extract was transferred to a cuvette and the optical density (OD) values at 645 and 663 nm were read using a spectrophotometer (Spectronic 1001) against a DMSO blank. For getting the yield, mature green chillies were harvested in 5 pickings at a 20-day time interval. Nitrogen contents in plants and residual soil nitrogen were determined according to the procedure provided by IITA (1975). The phosphate and potash contents from plant were estimated by the method of Lindner (1944). Similarly phosphate and potash content in plants and residues were determined with the method of Olsen et al. (1954) and Jackson (1973), respectively. The data of 3 years have been pooled and analysed statistically according to Pansey and Sukhatme (1978). The critical difference (CD) at P_= 0.05 probability was calculated considering the difference between various treatments.

Extraction of nematodes

The population of plant-parasitic nematodes was recorded from each bed before treatment as well as after terminating the experiment by processing representative soil samples by Cobb's sieving and decanting method along with Bearmann's funnel technique (Southey, 1986). An average of 5 counts were taken in each case to determine the population density of each nematode species.

Results and discussion

The results presented in the Table clearly revealed that the inoculation of the biological nitrogen fixers individually as well as in various combinations had a significant influence on growth, yield, and quality parameters of chilli in the presence of different recommended dose of inorganic nitrogen. Furthermore, the results indicated that plant height, number of primary branches, and pollen fertility increased significantly with all these treatments. In the treatment of dual inoculation of biofertilisers, such as *Azotobacter* and *Azospirillum*, along with 100% recommended dose of N and neem cake, yielded the highest height (77.62 cm), the highest number of primary branches/plant (14.8), and the highest pollen fertility (98.86%) compared to the untreated control (the height of plant = 46.27 cm, the number of primary branches = 6.0, and pollen fertility = 83.40%). A similar significant improvement in growth parameters due to inoculation with biological nitrogen fixers has been reported by Sengupta et al. (2002) in tomato (*Lycopersicon esculentum*). Organic fertilisers influence both yield and plant micronutrient contents and thus help sustain crop productivity (Mottaghian et al., 2008).

The number of green fruit/plant, green fruit yield/plant, and 100 green fruit/plant weight (g) increased significantly by all the treatments. The treatments of combined application of *Azotobacter* and *Azospirillum* together with neem cake and 100% recommended nitrogen recorded higher fresh fruit/plant green fruit yield and 100 green fruit weight. These parameters were found highest in this treatment compared to sole application of inorganic nitrogen (100% recommended dose) and neem cake. Among the single inoculations, the application of *Azospirillum* along with neem cake and 100% recommended nitrogen produced more yield than that of *Azotobacter*. In the absence of 100% recommended dose of inorganic nitrogen, Table. Effect of biofertilisers, nitrogenous fertiliser, and neem cake on the plant growth and yield.

Treatments	Plant height (cm)	Number of branches/plant	Percent pollen fertility (%)	Green fruits/ plant	Green fruit yield/plant (g)	100-green fruit/plant weight (g)
(T1) 100% recommended N + *Azotobacter* + Neem cake	67.50	10.5	96.03	82.7	223.46	264.37
(T2) 75% recommended N + *Azotobacter* + Neem cake	65.70	10.0	96.0	79.3	201.72	250.40
(T3) 50% recommended N + *Azotobacter* + Neem cake	64.58	9.1	94.60	77.0	190.17	240.57
(T4) 100% recommended N + *Azospirillum* + Neem cake	72.60	12.8	97.37	85.4	234.00	273.00
(T5) 75% recommended N + *Azospirillum* + Neem cake	70.84	12.0	96.50	83.0	230.50	270.00
(T6) 50% recommended N + *Azospirillum* +Neem cake	68.76	11.2	96.22	82.5	220.67	264.34
(T7) 100% recommended N + *Azotobacter* + *Azospirillum* + Neem cake	77.62	14.8	98.86	89.3	246.26	294.40
(T8) 75% recommended N + *Azotobacter* + *Azospirillum* + Neem cake	75.47	14.3	98.50	88.2	240.10	290.33
(T9) 50% recommended N + *Azotobacter* + *Azospirillum* + Neem cake	74.39	13.2	97.64	87.5	237.00	284.00
(T10) *Azotobacter*+ Neem cake	57.30	7.7	91.20	73.2	166.13	200.19
(T11) *Azospirillum* + Neem cake	60.26	8.3	92.17	75.4	173.64	223.65
(T12) *Azotobacter* + *Azospirillum* + Neem cake	62.44	9.0	93.30	77.0	180.07	231.00
(T13) Neem cake	55.54	7.0	90.44	70.2	150.14	186.52
(T14) 100% recommended NPK	53.72	6.8	88.29	78.0	194.50	244.73
(T15) Untreated Control	46.27	6.0	83.40	64.3	148.50	171.84
C.D. (P = 0.05)	5.67	0.73	6.77	6.23	15.46	19.34

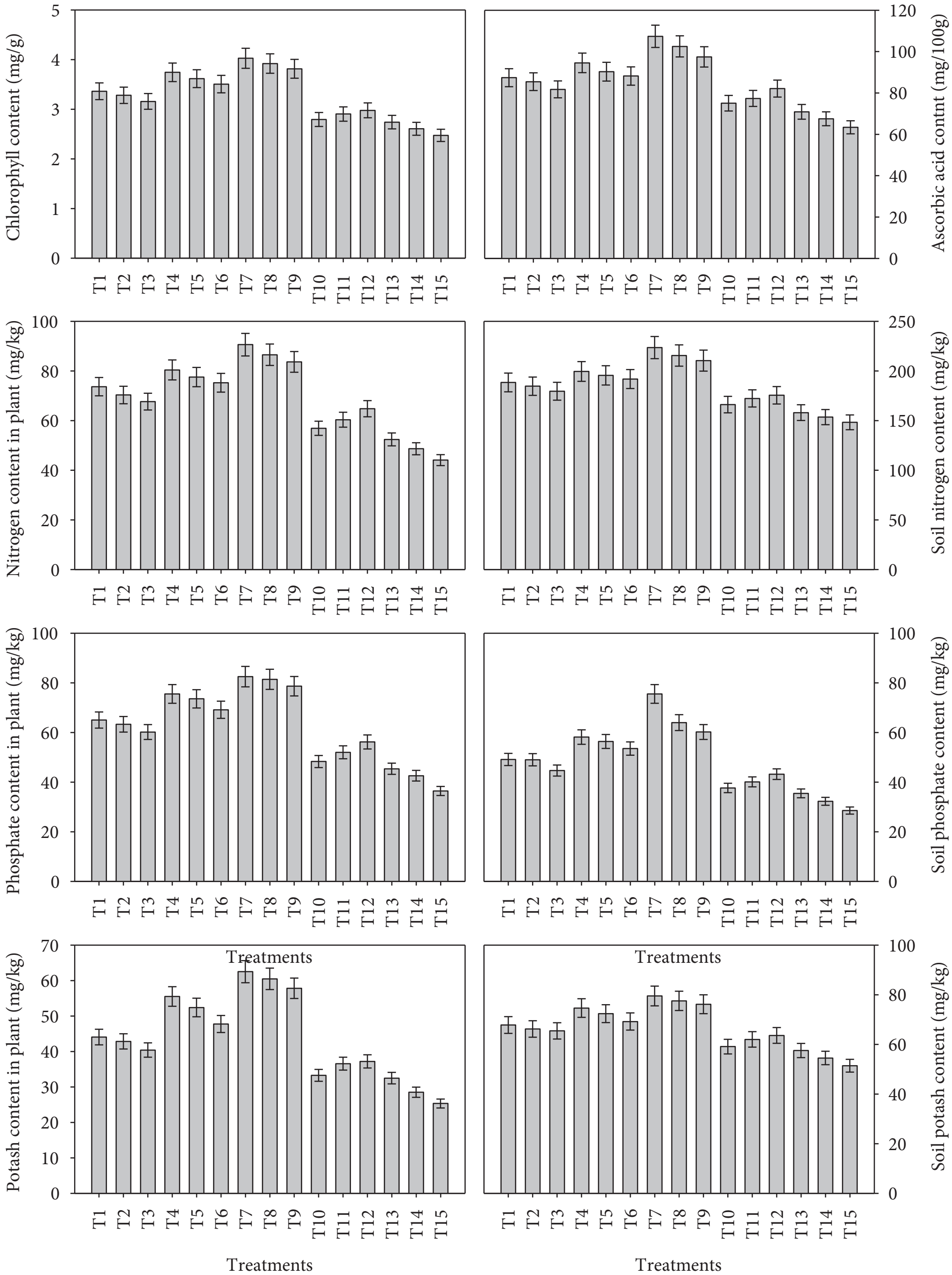
comparatively less green fruit yield was recorded from the beds treated with single and dual inoculation of *Azotobacter* and *Azospirillum*. Combined inoculation of the *Azotobacter* and *Azospirillum* always recorded better performance when compared with the single inoculation. This seems to be due to their synergistic effect. The increase in yield may be due to better root proliferation, more uptake of nutrients and water, higher plant growth, more photosynthesis, and enhanced food accumulation. Thus, associative nature of these biofertilisers helped to save large amounts of inorganic fertilisers in chilli. These findings are in conformity with those of Swain et al. (2003) and Wange and Kale (2003) in okra. Rajaee et al. (2007) also reported that free-living nitrogen fixing micro-organisms, such as *Azotobacter* and *Azospirillum*, enhanced root-development, increased water and mineral uptake, and produced plant hormones that might be responsible for growth of chilli plant. Similarly Malik et al. (2005) observed significant improvement in grain yield, chlorophyll content, and delayed flowering due to application of *Azotobacter* culture.

The chlorophyll content of leaf, ascorbic acid content, plant nitrogen, phosphate, and potash content, and residual soil nitrogen, phosphate, and potash content (Figure 1) were also increased significantly by individual as well as combined inoculation as compared to untreated control. In the dual inoculation of *Azotobacter* and *Azospirillum* with 100% recommended dose of nitrogen and neem cake recorded the highest chlorophyll content, ascorbic acid content, plant nitrogen, phosphate, and potash content, and residual soil nitrogen phosphate and potash content. The increase in nutrient content in soil was due to more retention of nutrients, especially P and K, which are added in the form of neem cake as well as biofertilisers. Long term application of organic manures (Tiwari et al., 2002) and biofertilisers (Bhunia et al., 2006; Kumar et al., 2009) were reported to improve soil organic carbon, available N, P, and K in soil thereby sustaining the soil health. Increased nutrient availability in organic matter-biofertiliser treatment was also due to increased dehydrogenase and phosphatase activity in the present study, an indication of increased soil biological activity (Parham et al., 2002). Ramesh et al. (2006) also reported the enhanced level of soil enzymatic activity; addition of organic manures promotes the recycling of nutrients in the soil ecosystem. The manurial effect of certain botanicals has also been observed by Knox et al. (2011). It is a well established fact that growth and yield is the outcome of complementary interaction between vegetative and reproductive growth of crop. Thus the better performance of growth parameters seems to promote yield attributes and thereby crop productivity (Bhati & Prasad, 2005). Gireesh (2009) also reported the direct relationship between photosynthetic pigment, such as chlorophyll content, and productivity of *Dunaliella salina*.

The population of plant-parasitic nematodes was found decreased in those beds treated with neem cake and biofertilisers, such as *Azotobacter* and *Azospirillum,* along with recommended dose of inorganic fertilisers (50%, 75%, and 100%). However, the maximum reduction was noted in beds treated with neem cake, *Azotobacter*, and *Azospirillum* together with 100% recommended dose of inorganic nitrogen (Figure 2). It may be due to the release of phenols from neem cake into amended soil. The phenolic compound decreased nematode population (Sitaramaiah & Singh, 1978; Singh et al., 1983). Biofertilisers are known to increase plant growth and subsequently induce resistance against nematodes (Durrant & Dong, 2004). Organically amended soil may change the physical properties of soil, which in turn may adversely affect the nematode behaviour, such as hatching, movement, and survival. The biofertilisers further improve the soil fertility through nitrogen fixation, thus supplement additional nitrogen, which might be detrimental to the population of nematodes. Phosphate is known to reduce soil pH, which has an adverse effect on nematode multiplication (Pant et al., 1983). Potash also showed an inhibitory effect on nematodes as reported by Gupta and Mukhopadhyaya (1971).

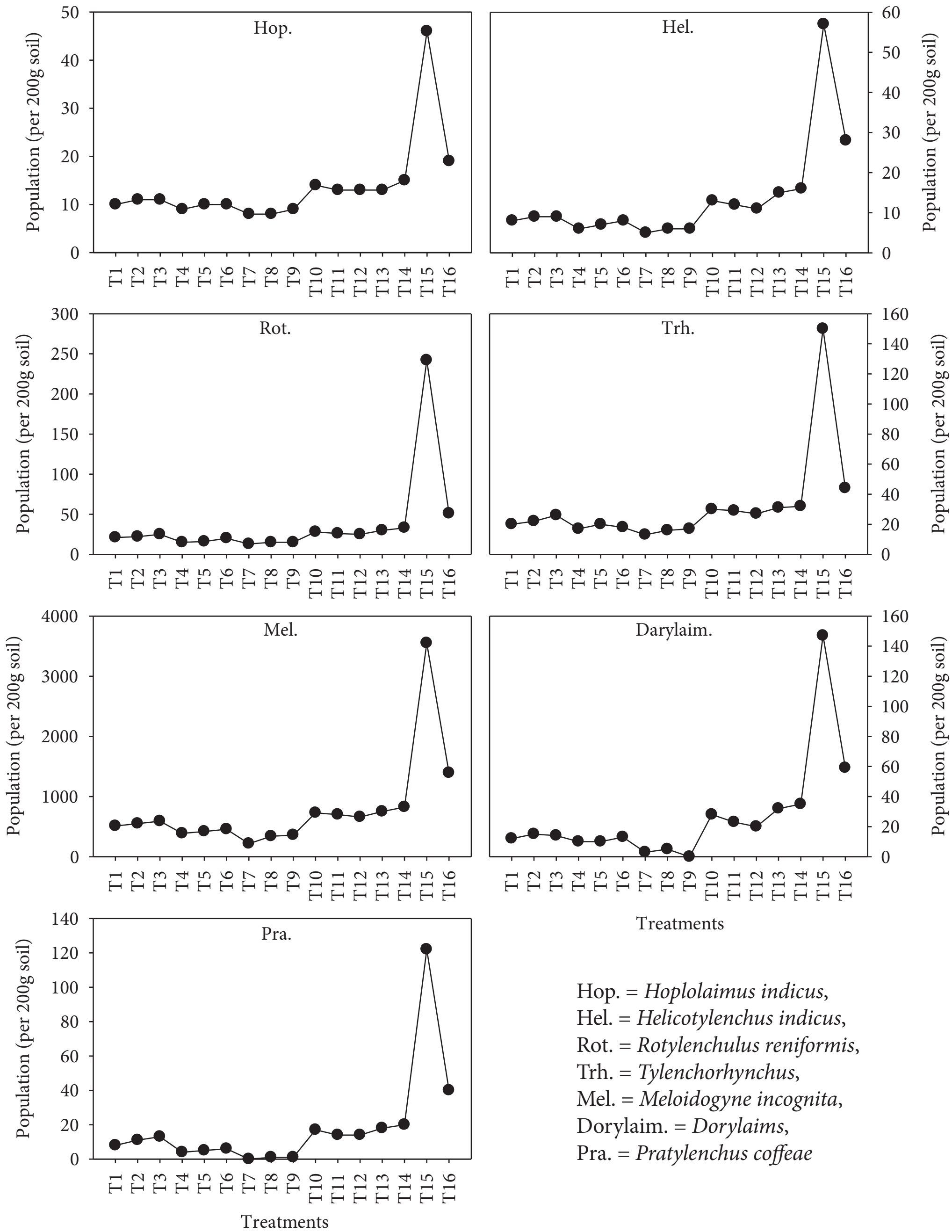
Conclusion and recommendation

Application of nutrients like neem cake, different nitrogen levels, and biofertilisers has a significant and vital effect on yield and quality attributes of chilli. The supply of various plant nutrients at an optimum level sustains the desired crop productivity by optimising the benefits from all sources in an integrated manner. The inference drawn from the present investigation



T1 = 100% recommended N + *Azotobacter* + Neem cake; **T2** = 75% recommended N + *Azotobacter* + Neem cake;
T3 = 50% recommended N + *Azotobacter* + Neem cake; **T4** = 100% recommended N + *Azospirillum* + Neem cake;
T5 = 75% recommended N + *Azospirillum* + Neem cake; **T6** = 50% recommended N + *Azospirillum* + Neem cake;
T7 = 100% recommended N + *Azotobacter* + *Azospirillum* + Neem cake; **T8** = 75% recommended N + *Azotobacter* + *Azospirillum* + Neem cake; **T9** = 50% recommended N + *Azotobacter* + *Azospirillum* + Neem cake;
T10 = *Azotobacter* + Neem cake; **T11** = *Azospirillum* + Neem cake; **T12** = *Azotobacter* + *Azospirillum* + Neem cake;
T13 = Neem cake; **T14** = 100% recommended NPK; **T15** = Untreated Control

Figure 1. Effect of biofertilisers, nitrogenous fertilisers, and neem cake on the chlorophyll content, ascorbic acid, N, P, and K from plant as well as from soil. Each treatment is a mean of 5 replicates. Bars show standard deviation.



T1 = 100% recommended N + *Azotobacter* + Neem cake; **T2** = 75% recommended N + *Azotobacter* + Neem cake; **T3** = 50% recommended N + *Azotobacter* + Neem cake; **T4** = 100% recommended N + *Azospirillum* + Neem cake; **T5** = 75% recommended N + *Azospirillum* + Neem cake; **T6** = 50% recommended N + *Azospirillum* + Neem cake; **T7** = 100% recommended N + *Azotobacter* + *Azospirillum* + Neem cake; **T8** = 75% recommended N + *Azotobacter* + *Azospirillum* + Neem cake; **T9** = 50% recommended N + *Azotobacter* + *Azospirillum* + Neem cake; **T10** = *Azotobacter* + Neem cake; **T11** = *Azospirillum* + Neem cake; **T12** = *Azotobacter* + *Azospirillum* + Neem cake; **T13** = Neem cake; **T14** = 100% recommended NPK; **T15** = Untreated Control; **T16** = Initial population of plant-parasitic nematodes.

Figure 2. Effect of biofertilisers, nitrogenous fertilisers and neem cake on the population of plant-parasitic nematodes infecting chilli plants. Each treatment is a mean of 5 replicates.

clearly stated that organics are effective alternatives as a source of macro- and micronutrients and have a potential to improve yield, and thus avoid costly chemical fertilisers. The bio-organic technology is based on eco-biotechnological approaches utilising the bio-transformation of energy rich and complex organic substances into bio-stabilised composed products. These organic substances provide nutrients for nematode antagonistic micro-organisms for arresting the population build-up of plant-parasitic nematodes. The biofertilisers, such as *Azotobacter* and *Azospirillum,* also produce growth promoting substances, increase soil fertility in terms of nitrogen, phosphorus, and potassium, and suppress the population of pathogenic nematodes. This type of experiment will definitely increase the interest amongst the related people to formulate ideas to develop organic agriculture, which sustains the crop production without harming our natural bio-resources.

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