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ORIGINAL RESEARCH

Influence of non-edible oil-cakes and their composts on growth, yield and Alternaria leaf spot disease in chilli

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Abstract

Purpose Raw and composted oil-cakes of neem, madhuca and simarouba were evaluated for their effect on plant growth, yield, and management of *Alternaria tenuissima* leaf spot disease, and rhizosphere microorganisms in chilli crop.

Method The oil-cakes were composted in simple pits containing a mixture (6:1:1) of individual oil-cake, soil and rice straw. Growth promotion and disease incidence were assessed in plants grown in soil amended with raw or composted oil-cakes of neem, madhuca and simarouba in pot and field. Rhizosphere microflora was also determined in all treatments.

Results Raw oil-cakes and their composts increased plant growth and yield and considerably decreased disease incidence and severity of *A. tenuissima* leaf spot in chilli grown in pot and field. The composted oil-cakes of simarouba were most effective in improving plant growth and yield and decreasing leaf spot disease in chilli, followed by madhuca and neem oil-cake compost. Fruit yield and vitamin C content were also high in simarouba compost. All composted oil-cakes increased beneficial microbial population in the rhizosphere, including phosphate solubilizers, free-living N_2 fixers and *Trichoderma* species. The compost amendment decreased *A. tenuissima* population in the soil at the same time.

Conclusion The growth promotion, yield increase and disease reduction in chilli were attributed to chemical compounds in oil-cakes and stimulation of beneficial microbes in the rhizosphere by raw or composted oil-cakes. This study demonstrated that composted non-edible oil-cakes could be used for soil amendment in place of agrochemicals to increase productivity, manage soil-borne diseases and improve soil health.

Keywords Leaf spot, Oil-cakes, Compost, Disease management, Yield improvement

Introduction

The non-edible oil-cakes, the byproducts of the biodiesel industry (Mishra and Mohanty 2018) are a valuable source of organic manure due to high nitrogen and mineral nutrient (Shivani 2011; Chaturvedi and Kumar 2012) and organic matter (OM) contents. This has generated a lot of interest in its application as a soil organic amendment (Lopes et al. 2009; Das et al. 2011). Among the non-edible oil-cakes, neem, pongamia and simarouba are being used directly as organic amendments to promote crop yield and minimize pest and pathogen incidences (Sahaa et al. 2010; Sharma et al. 2013; Singh and Prasad 2014). Certain phytochemicals in non-edible oil-cakes like phenols, tannins, alkaloids and organic acids have proven biocontrol activities (Alguacil et al. 2008; Saetae and Suntornsuk 2010; VasudhaUdupa et al. 2021). The oil-cakes are also applied in combination with other organic manures and inorganic fertilizers to enrich soil nutrient conditions depending upon the NPK requirement by the crop (Sinha et al. 2011; Parihar et al. 2015). However, there have been reports of toxic effects of the undecomposed oil-cakes to the crop plants in the field and hence oil-cakes are to be composted before soil amendment (Das et al. 2011; Chaturvedi and Kumar 2012). The composted oil-cakes of castor, jatropha and pongamia are shown to serve as a valuable source

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of materials alternative to chemical fertilizer(s) in providing nutritional requirements for good crop growth and yield increase (Chaturvedi et al. 2009; Gaind et al. 2009). The composts of organic wastes consist of stable end material with enhanced mineral nutrient availability that improves soil fertility, soil structure and disease suppression (Mehta et al. 2014, Sayara et al. 2020).

Chilli (*Capsicum annum* L., family Solanaceae), native to tropical America, is an important spice crop cultivated in India (Anonymous 1995). Chilli fruit is valued across the globe for its pungency due to the alkaloid capsaicin. The green chilli is also the source of vitamin A, C, B1, B2 (Saleh et al. 2018) and P (rutin) with immense pharmaceutical importance (Ganeshpurkar and Saluja 2017). The chilli crop is affected by fungal, bacterial and viral pathogens that reduce productivity (Agrios 2005; Yadav et al. 2017). Among the fungal pathogens, species of *Alternaria* cause die-back, leaf spot and fruit rot diseases in several crop plants (Narain et al. 2000; Jain et al. 2019). *Alternaria alternata* and *A. tenuissima* are seed-and soil-borne pathogens and incite leaf spot and blight disease symptoms in chilli crop (Narain et al. 2000; Azad et al. 2016). The leaf spot of chilli caused by *A. tenuissima* was first reported in China (Li et al. 2011) and subsequently in India (Azad et al. 2016). Efforts have been made to manage *A. tenuissima* disease in several crops by seed and soil treatment with fungicides and fertilizers (Azad et al. 2016). Since agrochemicals harm the environment and humans (Reshu and Khan 2012), there is an urgent need for safe and environment-friendly strategies to control plant diseases. One of the strategies is to amend the soil with crop residues, animal manure, green manure, or composts to improve soil organic matter content and fertility and decrease soil-borne disease incidences (Noble and Coventry 2005; Cavigelli et al. 2012). A literature review indicated detailed studies on the use of raw and composted oil-cakes of non-edible plant species on plant growth and yield and managing foliar fungal disease in chilli crop in the field (Abbasi et al. 2005; Chaturvedi et al. 2013) are lacking.

The objectives of the present study were to amend the soil with raw and composted non-edible oil-cakes of neem, madhuca or simarouba and study their influence on the plant growth and yield of chilli crop in the greenhouse and field. It was also aimed to determine the effect of the oil-cake amendment on the leaf spot disease caused by *A. tenuissima* in the field and the change in the rhizosphere microbial population in the soil. Also, there is no information on the effect of non-edible oil-cakes on the diversity of beneficial microbes and leaf spot pathogen in soil.

Materials and methods

Preparation of oil-cake composts of neem, madhuca and simarouba

Fresh non-edible raw oil-cakes of neem, madhuca, and simarouba were collected from the Biodiesel Production Unit of Biofuel Park located in Hassan, Karnataka, India. The above oil-cakes were composted separately by the simple pit method, with each pit (1×1.5×2 m) containing a mixture (6:1:1, w/w) of individual oil-cake, soil and rice straw. The mixture was allowed to compost for 90 days, with mixing at weakly intervals and adequate water sprinkles to maintain 70% moisture. After completion of incubation, composts of the respective oil-cakes were kept undisturbed in pits to cure for 30 days (Bernal et al. 1998) and then used for further experimentation. The raw and composted oil-cakes were determined for total organic carbon content (Official Methods of Analysis Association of Analytical Chemists (AOAC) method; Anonymous 1995), lignin (Goering and Van Soest 1970), total nitrogen (Kjeldahl method, (Krick 1950), total phosphorus (P, vanadomolybdate method, Jackson 2014) and total potassium (K, Flame photometer methods, Singh et al. 2007), and the C to N ratio was determined by dividing total C by total nitrogen for each sample.

Characterization of the leaf spot pathogen

Samples of leaves, twigs, and fruits of chilli exhibiting leaf spot disease symptoms were collected from the farmer's field near the GKVK campus, Bangalore during March 2017, brought to the laboratory, washed in tap water and observed microscopically for disease symptoms. The infected leaf sample was cut into 1-cm segments, surface disinfected with sodium hypochlorite (NaOCl, 1%, 1 min) and washed with sterilized distilled water, blotted to remove excess moisture, and placed on potato dextrose agar (PDA) or moistened blotters (Vasanthakumari and Shivanna 2013) and incubated in the dark at 23±2˚C for 48 to 72 hours. The mycelial fragments on incubated segments were isolated aseptically and transferred to PDA plates for purification. The pure culture of the pathogen was maintained on PDA slants and stored at 5˚C for further study.

The identity of the pathogenic fungal isolate was done based on morphological criteria (Sutton 1980). Further, the fungal DNA was isolated and sequenced by the method of White et al. (1990) based on the ITS1 and ITS2 regions of rDNA genes using ITS1 and ITS4 primers. The amplification was performed using a thermal cycler (Eppendorf Master cycler, USA) and the reaction involved an initial denaturation at 94 ˚C for 1 min followed by 35 cycles of 94 ˚C for 1min, 55 ˚C for 30 s annealing and elongation at 72 ˚C for 1min. and the final extension was set at 72 ˚C for 12 minutes. The amplified DNA was sequenced, and the sequence was analyzed by the online NCBI BLAST tool program (http://www.ncbi.nib.gov/blast). The DNA sequences of the pathogenic isolate were submitted to the NCBI Gen Bank, and the accession number was obtained.

Six isolates of the pathogen that were obtained from diseased leaf segments were cultured on PDA for 5-7 days. The spore suspension (5µl, 1×10^5 spores ml^{-1}) of each isolate was prepared and prick-inoculated to detached surface disinfected, apparently healthy chilli leaves placed on moist blotters in Petri plates. The artificially inoculated leaves were incubated in the dark in a growth chamber (70-80%, RH) for 5-8 days and observed for the disease symptoms. The disease symptoms and spores produced were used for confirming the identity of fungal pathogen.

Experimental set up for determining the growth, yield and leaf spot disease

Pot experiment

The mass inoculum of *A. tenuissima* was obtained by culturing the pathogen in the autoclaved sorghum grains (Vasanthakumari and Shivanna 2013). The autoclaved grains completely colonized by the pathogen were air-dried under ambient conditions and ground (1-mm particle size) with a blender. The colony-forming units (cfu g^{-1}) of the pathogen were determined. Chilli seed samples (Guntur var. G4 with 100 % seed germinability) without the seed-borne incidence of *A. tenuissima* (Anonymous 1995) were selected for the study. The potting medium (one kg) was prepared by mixing red loam soil and sand (1:1) and amended (0.5%) with raw oil-cake or composted oil-cake. The pot experiment was conducted in a completely randomized design (CRD) with three replicates in each treatment. Each potting cover (one kg capacity) received the potting medium (1000 g) amended with oil-cake (0.5%) and sorghum grain inoculum (@ 0.1%) of *A. tenuissima*. Potting covers that received only fungal colonized autoclaved sorghum grain served as positive control and those without any oil-cake or pathogen inoculum served as the negative control. The experiment was conducted for four weeks from December 2016 to January 2017 and repeated during March-April 2017 in the greenhouse (average RH 80 %; temperature 25-28°C, solar illumination, with regular watering). The disease incidence (DI, %) and severity (DS, %) in plants were recorded (Vasanthakumari and Shivanna 2013). Chilli seedlings (5 per replicate) were removed from the soil with intact roots, washed in running tap water and data on length, and fresh and dry biomass (hot-air oven at 60 ± 2 °) of seedlings were collected at an interval of seven days for four weeks.

Field experiment

Field trials at the experimental field (N13˚5 ' 1 ' ' and E77˚34' 38') of the Department of Forestry and Environmental Science, UAS, GKVK, Bangalore) were conducted (2017-18 and 2018-19) to determine the influence of three non-edible oil-cakes of neem, madhuca and simarouba and their composts to increase plant growth and *Alternaria* leaf spot disease in chilli plants. The experimental field contained red sandy loam soil (pH 6.78) and experienced an average rainfall of 229.5 mm and an average RH of 86% and the temperature ranging from 20.1˚C to 31.2˚C during the experiment period. The land was prepared to excellent tilth with one deep ploughing followed by three harrowing. The side bunds were prepared around each plot for individual treatments. Plots were arranged in the Randomized Complete Block Design (RCBD) with three replicates per treatment. The individual micro plots (1.5×1.5 m, 3 sqm) were leveled and ridges and furrows (45 × 45 cm distance) were made (three rows per micro plot). The raw or composted oil-cakes were added separately to the soil @10 tons ha^{-1} during the transplantation. Inorganic fertilizer (100:75:50 kg of N: P: K) in all treatment plots were also applied separately. Seeds of chilli var. G4 were sown in nursery portrays containing moist coco peat and raised for 30 days, and apparently, healthy chilli seedlings were transplanted in each row at a distance of 45 cm (15 plants/plot). The sorghum grain inoculum (1 g) of the pathogen was placed near the seedling roots at the time of transplantation and spore suspension of *A. tenuissima* (2×10^{-2} spores ml^{-1}) in sterile water was sprayed on foliage at 10 days after transplant to ensure the disease development. Plants were grown for 120 days in the field and irrigated regularly. Plants in one experimental plot (Plot A) were used for collecting data on pre-and post-emergence mortalities and DI and DS due to *A. tenuissima*. Plants in another plot (Plot B) which were used to determine the plant growth and biomass of plants and fruits at different growing stages, which required plant harvesting. Data on shoot length, fresh and dry biomass of chilli plants were collected at an interval of 30 days and data on the number of fresh and dry biomass of ripe fruits were collected at 90 and 135 days after transplanting (DAT). Chilli fruits in Plot A plants were harvested twice, at 105 and 120 DAT and assessed for DS. The vitamin C content of green chilli was determined (Papuc et al. 2001) by titration with 2,6, dichlorophenol indophenols (DCPIP, 0.1%). Fruits that exhibited 0, 50 and 100% DI and DS were collected separately and seeds from fruits of the same disease category in each treatment were pooled to obtain samples to determine the seed-borne incidence of *A. tenuissima* and seed germinability (Anonymous 1995).

The field rhizosphere samples were collected from the soil region (4-6 cm) adjoining the plant root at 120 DAT by piercing with a sterile cork borer (10 cm length, three cm-dia). The rhizosphere soil sample (1 g) towards the borer base was collected and subjected to soil dilution plating and soil suspension spread on the culture media (Cindy et al. 2013). The free-living nitrogen-fixing and phosphate solubilizing rhizosphere bacterial species were enumerated at 10^{-6} dilution on King's B agar (Himedia), Waksman No 77 and Pikovaskay's agar media. The nutrient agar medium was used for enumerating the total rhizosphere bacterial isolates. The fungal species such as *Trichoderma* (biocontrol agent) and *A. tenuissima* (pathogenic fungus) were enumerated at 10^{-4} dilution in PDA media.

Statistical analysis

The pot and field experiments were arranged in the Completely Randomized Design (CRD) and Randomized Complete Block Design (RCBD), respectively. The data of field experiments are subjected to the homogeneity of trials by analysis of variance (ANOVA, $P \leq 0.05$). Since trials were homogenous, the data of two trials were averaged and the means of treatments were compared by the least significant difference (LSD, $P \leq 0.05$) and Duncan's multiple range test (DMRT, $P \leq 5\%$). Further, the data of different parameters in the field experiment were analyzed by the Pearson correlation coefficient.

Results and discussion

The organic amendments used for good plant growth and disease control depend on the raw materials and their chemical components (Termorshuizen et al. 2004; Bonanomi et al. 2020). Simarouba raw oil-cake contains high total organic carbon (53.76%) followed by madhuca (49.1), and neem had the lowest. The lignin content is high in madhuca raw oil-cake (31.3%) which is followed by neem and simarouba. The high organic carbon and lignin contents in raw oil-cakes reduced considerably following composting (Table 1). Lignocellulose is a complex material comprising cellulose, hemicellulose and lignin (Perez et al. 2002; Zoghlami and Paes 2019), and the degree of lignin to degradation depends on the type of microbial enzymes. All the raw oil-cakes used in the study are acidic and composting of these oil-cakes resulted in neutral pH (6.68 to 7.02). The increase in pH value coincided with the production of ammonia gas due to the degradation of proteins during decomposition (Sharma et al. 2008; Bohacz 2019). On the other hand, the composted oil-cakes' electrical conductivity (EC) was higher than the raw oil-cakes. The elevation in EC of composted oil-cakes could be due to the increase in the available minerals, in the ionized form (Wong et al. 2001; Caceres et al. 2006). The total N content was high in the neem oil-cake compost, while P and K contents were high in madhuca oil-cake compost (Table 1). However, the C/N ratio was high in raw madhuca oil-cake and low in neem oil-cake (Table 1) but after decomposition, C/N ratio was lowered to 15.68-19.35, in all the oil-cakes. the C/N ratio decreases over composting time increased (Tibu et al. 2019). With a low C/N ratio, the compost is suitable for plant growth and hence recommended for soil application (Bernal et al. 1998; Al-Bataina et al. 2016).

Influence of raw and composted oil-cakes on plant growth, yield and leaf spot disease

Characterization of leaf-spot pathogen

The chilli leaf spots are small circular, brown, and necrotic on the leaf lamina, enlarging into irregular spots with sporulation of *A. tenuissima* in concentric rings. The observed symptoms were correlated with the previous report of chilli leaf spot by *A. tenuissima* (Azad et al.

Table 1 Physicochemical properties of raw and composted oil-cakes and field soil used for pot experiment

Oil-seed cakes	pH	EC	OC (%)	N (%)	C/N	P (%)	K (%)	Lignin (%)
Raw neem oil-cake	6.19±0.2[1]	1.18±0.02	20.67±1.3	3.9±0.3	5.3±0.3	0.36±0.02	1±0.02	21.5±0.3
Raw madhuca oil-cake	6.74±0.3	0.71±0.03	49.1±1.2	1.5±0.2	32.73±0.3	0.29±0.03	0.6±0.03	31.3±0.3
Raw simarouba oil-cake	6.76±0.2	0.82±0.03	53.76±1.3	7.1±0.3	7.6±0.3	0.28±0.04	0.4±0.03	8.5±0.3
Comp[2]. neem oil-cake	7.02±0.3	2.43±0.02	35.3±1.2	2.5±0.2	15.68±0.2	0.28±00.3	1.73±0.02	18.3±0.3
Comp. madhuca oil-cake	6.9±0.4	1.95±0.03	43.6±1.3	2.4±0.3	18.16±0.2	0.32±0.03	2.3±0.02	29.8±0.3
Comp. simarouba oil-cake	7.01±0.2	2.3±0.03	32.9±1.3	1.7±0.3	19.35±0.3	0.26±0.03	0.91±0.03	6.3±0.3
Field Soil	6.78±0.3	0.23±0.03	-[3]	-	-	0.19±00.3	0.93±0.04	-

[1]Mean ± SE ; [2] Composted; [3]Not determined

2016). Enlarged spots coalesced to produce blight symptoms when leaves withered. On the incubated leaves and PDA, the pathogen produced greyish mycelial growth and sporulation. The conidia (6-8) in the chain were produced and each conidium contained 2-6 transverse septa and 0-2 longitudinal septa. All the above characteristics suggested that the casual organism of leaf spot disease is *Alternaria tenuissima* L. Based on the sequence of rDNA-ITS regions of the highly infective isolate, which showed 100% similarity with that of *Alternaria tenuissima* (Acc. No: MF380833.1), the identity of the pathogen was confirmed as *A. tenuissima* (Acc. No: MN900519). The artificial inoculation of chilli leaves with *Alternaria tenuissima* suggested its high pathogenic ability. *Alternaria tenuissima* is a well-studied pathogen capable of causing leaf spot disease in various crop plants (Rathod and Chavan 2010; Sharma et al. 2012), including chilli (Zheng and Wu 2013; Azad et al. 2016).

Effect of oil-cakes on plant growth and yield

Among the raw oil-cakes, neem improved plant growth and biomass, while that of madhuca and simarouba inhibited plant growth ($P \leq 0.05$) when compared to the control without raw oil-cakes in the pot and early days of field experiment (Table 2, Fig. 2). The raw oil-cakes of simarouba and madhuca caused an adverse effect on seedlings. The reduction in seedling growth could be attributed to the phytotoxic effect of compounds in raw oil-cakes. Prasad et al. (2005) reported that the toxic effect was due to quassinoids in simarouba oil-cake. Madhuca oil-cake along with simarouba also contained a phenolic compound 2,4-di-tert-butylphenol (VasudhaUdupa et al. 2021) which was phytotoxic to the plant system (Halim et al. 2017). Certain non-specific phytotoxic compounds have also been reported in undecomposed organic amendments, which affected root growth (Lehmann et al. 2011; Ling et al. 2016). These phytotoxic compounds may liberate when applied to the soil by native saprophytic microbes through oil-cake mineralization (Buchmann et al. 2015). The prier composting of oil-cakes before soil amendment results in the degradation of phytotoxic compounds due to microbial action or other physico-chemical factors (Chaturvedi et al. 2013; VasudhaUdupa et al. 2017a; Jagadabhi et al. 2019). The previous study was made to eliminate phytotoxicity (polyphenols and fatty acids) of olive mill waste and sugarcane processing industry through composting and solved the environmental impact of this waste (Kundan et al. 2015; Luz et al. 2020). The composted simarouba cake was superior to madhuca and neem in increasing ($P \leq 0.05$) shoot and root length, a number of branches and fresh and dry biomass of shoot and plant growth response in the pot and field experiment was observed (Table 2, 3 and 4; Fig. 1). This could be due to the presence of higher gibberellic acid (GA_3), a plant growth hormone concentration (1007 mg kg^{-1}) in simarouba cake compost compared to the neem (219 mg kg^{-1}) and madhuca (118 mg kg^{-1}) cake compost (Va-

Table 2 Effect of amendment of soil with raw and composted oil-cakes of neem, madhuca and simarouba on the shoot and root length and number of branches in 3-week-old chilli plants in the greenhouse[1]

Sl. No	Treatments	Shoot length (cm)	Root length (cm)	Number of branches[2]
1	Control (negative)[3]	18.17±0.31c[4]	19.18±0.23 b	11±0.2 b
2	Control (positive)[5]	13.38±0.22a	13.75±0.15 a	7±0.1 a
3	Raw neem oil-cake	19.15±0.09 c	20.13±0.07 b	14±0.07 c
4	Raw madhuca oil-cake	15.38±0.14b	14.41±0.06 a	8±0.15 a
5	Raw simarouba oil-cake	12.03±0.11a	13.45±0.13 a	12 ±0.08b
6	Comp[6]. neem oil-cake	40.3±0.09 e	43.45±0.16 d	18±0.1 d
7	Comp. madhuca oil-cake	30.13±0.06 d	34.15±0.07c	15±0.07c
8	Comp. simarouba oil-cake	45.15±0.17 f	48.36±0.06 e	20±0.08 e

[1]Experiment was conducted in completely randomized design; [2] Includes both primary and secondary branches; [3]Control without pathogen and oil-cakes; [4] Mean±SE followed by same letters are not significantly different according to DMRT ($P \leq 0.05$); [5]Control with the pathogen and without oil-seed cakes; [6]Composted

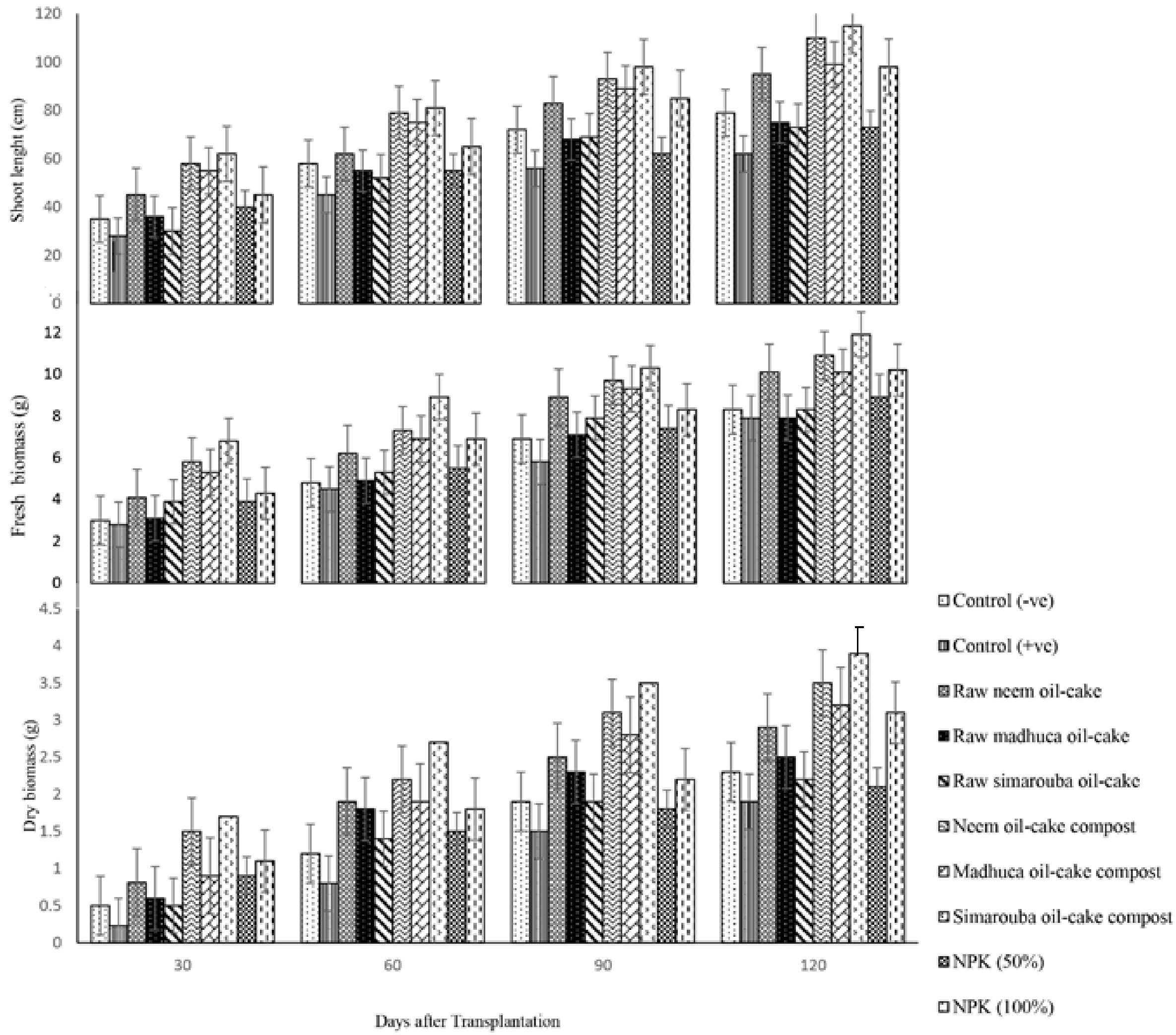

Fig. 1 Effect of amendment with raw and composted oil-cakes of neem, madhuca and simarouba on shoot length and fresh and dry biomass of chilli plants grown in the field

sudhaUdupa et al. 2017b). The presence of phytohormones in compost from municipal solid waste and tannery waste (Ravindran et al. 2016; Klimas et al. 2016) have linked to the numerous physiological processes in plants (Verma et al. 2016) and disease suppression of soil-borne pathogens (Morales-Corts et al. 2018). The gibberellic acid has several effects on plant development by stimulating rapid stem and root development, leaf area, chlorophyll content, fresh and dry biomass of the plant (Zang et al. 2016). The growth promotion by the compost of oil-cakes compared to raw oil-cakes also linked to the presence of humus substances which are the previously known growth-enhancing and disease suppressing constitute of the compost (Bonanomi et al. 2020).

In the 12-week field experiment, the raw oil-cakes

Table 3 Effect of soil amendment with raw and composted oil-cakes of neem, madhuca and simarouba on the number of chilli fruits[1] harvested at different intervals in the field[2]

TreatmentS	Days after transplantation			
	90	105	120	135
Cont. (-ve)[3]	10±0.5[4]	15±0.4	18±0.5	21±0.7
Cont. (+ve)[5]	5±0.5	11±0.5	13±0.7	15±0.7
Raw neem oil-cake	19±0.3	22±0.7	27±0.8	31±0.5
Comp[6]. neem oil-cake	21±0.5	27±0.6	33±0.5	38±0.3
Raw madhuca oil-cake	9±0.3	12±0.3	15±0.6	17±0.7
Comp. madhuca oil-cake	27±0.4	35±0.5	39±0.7	43±0.3
Raw simarouba oil-cake	13±0.3	17±0.7	19±0.5	22±0.3
Comp. simarouba oil-cake	30±0.3	38±0.6	44±0.5	48±0.7
NPK (50%)[7]	8±0.5	12±0.5	15±0.6	24±0.5
NPK (100%)[8]	15±0.3	18±0.7	24±0.5	28±0.7
$LSD^{9}_{0.05}$	1.9			
$LSD_{0.01}$	2.34			

[1]Includes both ripened red as well as green fruits of all ages; [2]Experiment was conducted in randomized complete block design. Mean values were compared with LSD (P=0.05, 0.01); [3]Control without pathogen and oil-cakes; [4]Mean±SE of three replicates each with 15 plants (5 plants/pit); [5] Control with pathogen and without oil-cake; [6] Composted; [7]NPK (50:25:25) @ 50% of recommended dose; [8] NPK (100:50:50) @ 100% of recommended dose; [9] Least significant difference (P=0.05, 0.01)

of madhuca and simarouba have shown increased (P≤0.05) plant growth and biomass with the increase in the number of DAT, when compared to the control (Fig. 2). A lot of information is available on the degradation of organic materials in soil due to autochthonous bacterial and fungal species (Saadi et al. 2007; Sellami et al. 2008). Compared to the raw oil-cakes, the composted oil-cakes enhanced plant growth and biomass to a high level, particularly that of simarouba caused growth-increase greater than that of neem and madhuca. These increases were visible in plants at 60 DAT and afterwards. The treatment with NPK @100% also contributed significantly (P≤0.05) to plant growth and biomass increase, but it was not more than composted oil-cake treatments at 90 or 120 DAT. The observed plant growth promotion in compost treatment could be due to the increase in mineral nutrient availability in the soil (Rady et al. 2016) following mineralization by microbes and their absorption by plants (Meera et al. 1994; Mehta et al. 2014). The increase (P≤0.05) in fruit number was also evident in the composted oil-cakes as compared to the raw oil-cakes and NPK fertilizer treatments (Table 3). The high fruit yield was observed with simarouba oil-cake compost, followed by madhuca and neem oil-cake composts, where it was increased by two times when compared with NPK treatment @100% (Table 3). This indicated that composts of simarouba and madhuca could be a good alternative to NPK in respect of chilli yield (Table 4). The significant increase in fruit length correlated positively (R = 0.79) with vitamin C content of chilli fruits when composted simarouba cake was amended to the soil, which was followed by madhuca, all raw oil-cakes and NPK treatments. A similar observation was made in common beans in respect of yield improvement when compost was applied to the field (Rady et al. 2016).

Similarly, fresh and dry biomass of chilli fruits was

Table 4 Effect of soil amendment with raw and composted oil-cakes of neem, madhuca and simarouba on attributes of chilli fruits harvested at different intervals[1] in the field

Fruit characters/DAT[2]	Control		Neem		Madhuca		Simarouba		NPK	
	ve[3]-	ve[4]+	Raw	Comp[5]	Raw	Comp	Raw	Comp	NPK (50%)	NPK (100%)
Fruit length (Excluding pedicel in cm)										
90	7.3±0.1	6.6±0.06	10±0.05	13.5±0.08	9.2±0.1	12.3±0.1	9.5±0.1	13.8±0.06	8.3±0.1	10.8±0.1
135	7.9±0.08	7.2±0.1	11±0.1	13.9±0.1	10.3±0.1	13.0±0.1	10.4±0.1	14.3±0.1	8.9±0.1	11.2±0.1
LSD[6] $_{(P\leq0.05)}$	0.32									
LSD $_{(P\leq0.01)}$	0.44									
Fresh biomass (g) / fruit										
90	3.9±0.1	3.1±0.08	6±0.1	7.9±0.06	5.98±0.1	7.2±0.07	5.84±0.1	8.2±0.1	5.9±0.06	6.2±0.08
135	2.8±0.1	2.3±0.1	2.7±0.07	2.79±0.1	2.75±0.1	2.85±0.1	2.64±0.1	2.93±0.1	2.73±0.1	2.8±0.09
LSD	0.3									
LSD	0.40									
Dry biomass (g) / fruit										
90	0.64±0.02	0.51±0.03	0.98±0.01	1.2±0.04	0.92±0.05	1.12±0.03	0.94±0.02	1.42±0.05	0.98±0.04	1.04±0.04
135	0.49±0.03	0.42±0.04	0.66±0.02	0.81±0.04	0.64±0.03	0.81±0.02	0.68±0.02	0.84±0.02	0.67±0.01	0.78±0.04
LSD	0.17									
LSD	0.24									
Vitamin C content (mg) / g of fruit										
90	0.99±0.05	0.84±0.04	1.2±0.02	1.3±0.04	0.98±0.03	1.2±0.05	1.4±0.02	1.8±0.03	0.86±0.03	1.2±0.04
LSD	0.12									
LSD	0.23									
Fruit yield/plant (g)										
90	39±0.3	15.5±0.5	114±0.4	165.9±0.5	53.82±0.3	194.4±0.5	62.4±0.6	246±0.5	47.2±0.5	93±0.6
135	58±0.5	8.34±0.6	83.7±0.6	102.6±0.6	45.9±0.3	120.4±0.4	57.2±0.5	139.2±0.7	64.8±0.6	78.4±0.5
LSD	0.9									
LSD	1.7									
Fruit yield (t/ha)										
90	1.9±0.6	0.7±0.6	5.7±0.7	8.2±0.7	2.6±0.5	9.7±0.6	3.1±0.5	12.3±0.5	2.3±0.6	4.6±0.6
135	2.9±0.6	0.4±0.5	4.1±0.5	5.1±0.5	2.2±0.4	6.0±0.6	2.8±0.6	6.9±0.7	3.2±0.7	3.9±0.6
LSD	1.0									
LSD	1.6									

[1]Experiment was conducted in a factorial design; [2] Days after transplantation;[3]Control without pathogen and oil- cakes;[4]Control with pathogen and oil-cakes;[5] Composted;[6]LSD values (P=0.05 and P0.01) for comparing means±SE under each treatment are given in the bottom of the column

also improved considerably due to amendment with composted oil-cakes of simarouba, followed by neem and madhuca; and all three raw oil-cakes caused similar effects (Table 4). The food waste compost application to the soil was also shown to increase the yield of cabbage, cauliflower and radish, which was attributed to the improvement of relative water content and decreased electrolyte leakage (Kumari et al. 2020). The compost is reported to support plant growth-promoting rhizobacteria that are well known for improving plant growth and vigor (Castano et al. 2011). Both the raw and composted oil-cakes stimulate the beneficial microbial consortia in the rhizosphere, where they degraded various components in the raw cakes to easily available form of nutrients, which form the source of nutrients for the rhizosphere microbes (Tiyagi et al. 2001; Zhao et al. 2018). The mechanism of growth promotion by these microbes was attributed to the production of plant growth-promoting substances, including phytohormones (Olanrewaju et al. 2017; Kudoyarova et al. 2019), phosphates in the soluble form (Deepa et al. 2010) or fixation of atmospheric nitrogen (Igiehon and Babalola 2018). The chilli plants grown in the soil amended with biocontrol agents not only increased the plant growth, fruit biomass and number but also increased the fruit pungency and intense red coloration of ripened fruits (Vasanthakumari and Shivanna 2013).

Effect of oil-cakes on leaf spot disease of chilli

The disease incidence (DI) and disease severity (DS) of the leaf spot disease reduced significantly ($P \leq 0.05$) due to soil treatment with either the raw or composted oil-cakes. The DI and DS were predominantly reduced when treated with raw oil-cakes of madhuca and neem followed by simarouba and the composted oil-cakes were next in effectiveness. This could be due to the presence of a certain antifungal compound(s) in the raw-cakes or generation of ammonia during decomposition in the soil which is detrimental to the establishment of the pathogen and expression of disease symptom (Saetae and Suntornsuk 2010; VasudhaUdupa et al. 2021). However, composting reduced the effect of such compounds in raw oil-cakes, with an increase in the period after the oil-cake amendment and the growth stage of plants. A similar observation was made for the above oil-cake samples and their composts in sorghum plant growth (VasudhaUdupa et al. 2017b). The raw and composted madhuca oil-cakes were highly effective in decreasing the DI and DS when compared to neem and simarouba counterparts in both pot and field conditions (Figs. 1 and 3). It is also interesting to note that the DI and DS were decreased by 50%, irrespective of the raw oil-cake/compost treatment (Fig. 3). There is no report of the control of leaf spot disease-causing pathogen *A. tenuissima* in chilli by soil treatment with non-edible oil-cakes, in either the raw or compost form. The enhanced plant growth and reduced *A. tenuissima* leaf spot disease incidence and severity in chilli could be attributed to the increased availability of nutrients and presence of the antimicrobial compounds in raw oil-cakes or the activation of beneficial microbial consortia in the rhizosphere of chilli plants grown with either raw or composted oil-cakes. This finding agrees with those of previous reports (Bahramisharif et al. 2013; Tewoldemedhin et al. 2015). Neher et al. (2017) showed that the mature compost of wood chips/bark suppressed the soil-borne pathogen *Rhizoctonia*. The activation of beneficial or antagonistic microbes by oil-cake or compost amendment to soil could be attributed to the increased availability of nutrients in the easily available form (Hadar and Papadopoulou 2015; De Corato 2020). Composts are also shown to suppress plant diseases through the combination of physico-chemical (mineral nutrients, organic matter, pH, moisture) and biological (inhibiting microbial population, production of antibiotics, lytic and other extracellular enzymes) mechanisms (Boulter et al. 2000; Jeanine et al. 2002; Garbeva et al. 2008). Some examples of antagonistic microbes activated by the amendment of composts, raw oil-cakes or other organic amendments include species of *Bacillus* and *Pseudomonas* sp. (Bonanomi et al. 2018). The disease reduction due to these microbes in soil has been studied extensively (Pal and Gardener 2006; Begum et al. 2010). Another possible explanation for the reduced leaf spot disease incidence could be attributed to the induction of systemic resistance by beneficial microbes associated with the roots or rhizosphere (Meera et al. 1994) when the soil was amended with raw/composted oil-cakes (Hoitink and Boehm 1999; Antoniou et al. 2017). The treatment of NPK @100% to the soil was also useful in decreasing disease, but NPKs@50% treatment failed to decrease the disease (Fig. 3). Probably, low NPK content predisposed the plants to infection. This indicated that the proper dose of nutrient supply to the soil is associated with healthy plant growth (Datnoff et al. 2007; Jayawardana et al. 2016).

Seeds collected from plants grown with raw and

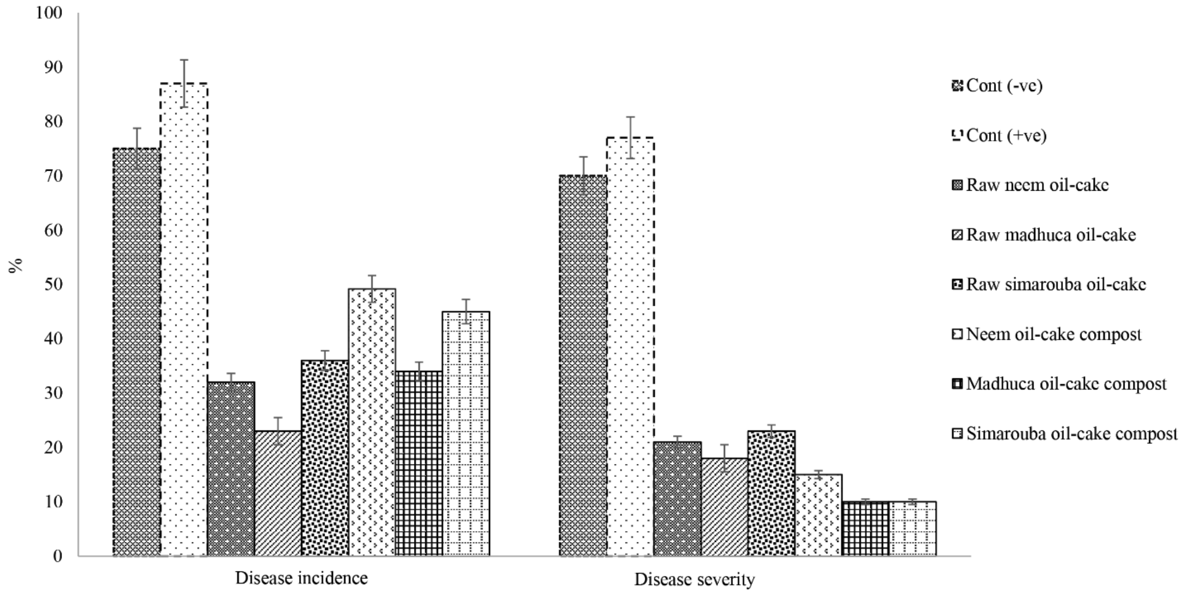

Fig. 2 The incidence (%) and severity (%) of leaf spot disease caused by *Alternaria tenuissima* in chilli grown in potting medium amended with raw or composted oil-cakes of neem, madhuca and simarouba

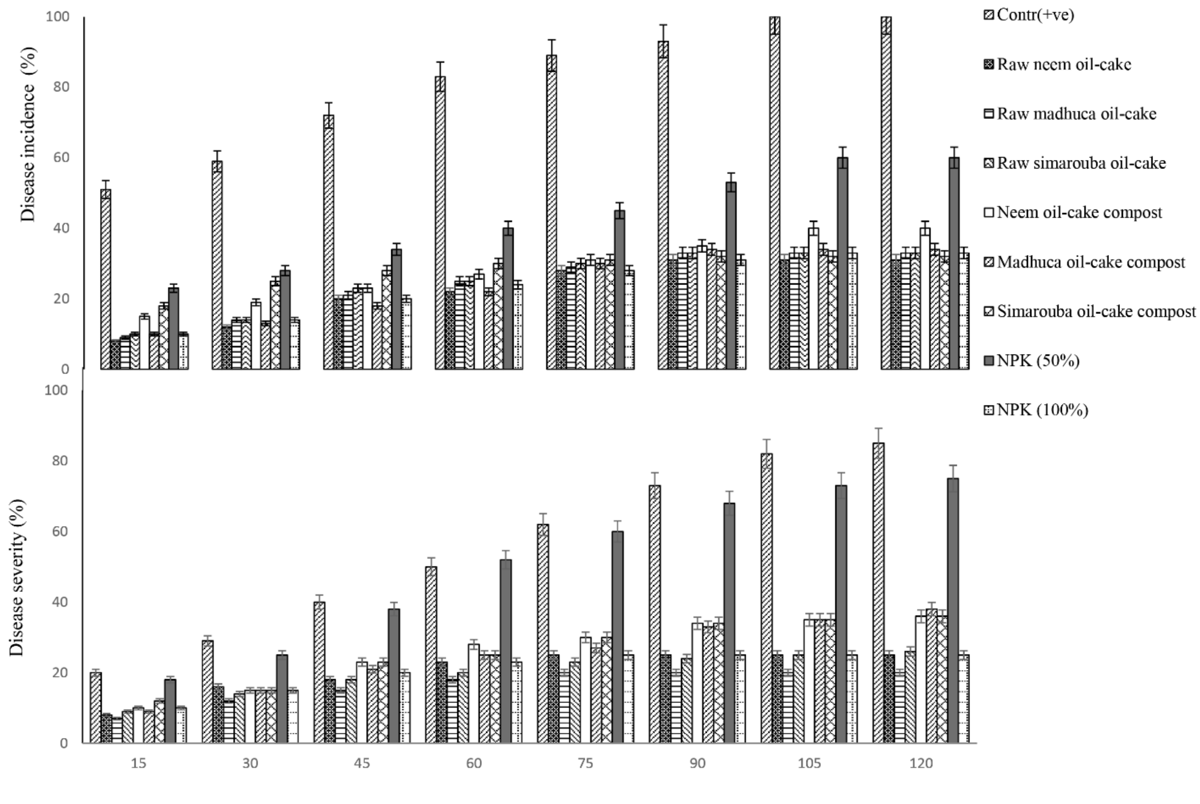

Fig. 3 Effect of soil amendment with raw or composted oil-cakes of neem, madhuca and simarouba on the incidence and severity (%) of leaf spot disease in chilli caused by *Alternaria tenuissima* in the field

composted oil-cakes and NPK @100% treatments germinated normally (95%), while in NPK @50% treatment, it was reduced to <80%. The incidence of *A. tenuissima* in seeds of plants grown in different treatments, except NPK@50%, reduced significantly (P≤0.05) (Fig. 4). High reduction in seed-borne pathogen incidence in raw and composted oil-cake treatments suggested the enhanced resistance in plants that are associated with oil-cakes. Reduced seed-borne infection in chilli was also reported upon soil treatment with biocontrol agents (Vasanthakumari and Shivanna 2013). Studies have shown that systemic resistance was induced in tomato plants grown in association with microflora or abiotic factors in the compost (Hoitink et al. 2006; Bahramisharif and Rose 2019).

The present study revealed that the raw oil-cakes reduced the leaf spot disease but failed to improve yield due to phytotoxicity in contrast to the composted oil-cakes which successfully reduced the leaf spot disease and increased the yield, as well. Similar observations were also reported with raw or composted plant residue amendment in soil and the resultant disease reduction followed by yield improvement (Mehta et al. 2014; Kundan et al. 2015; Luz et al. 2020).

Effect of oil-cakes on rhizosphere microbial density

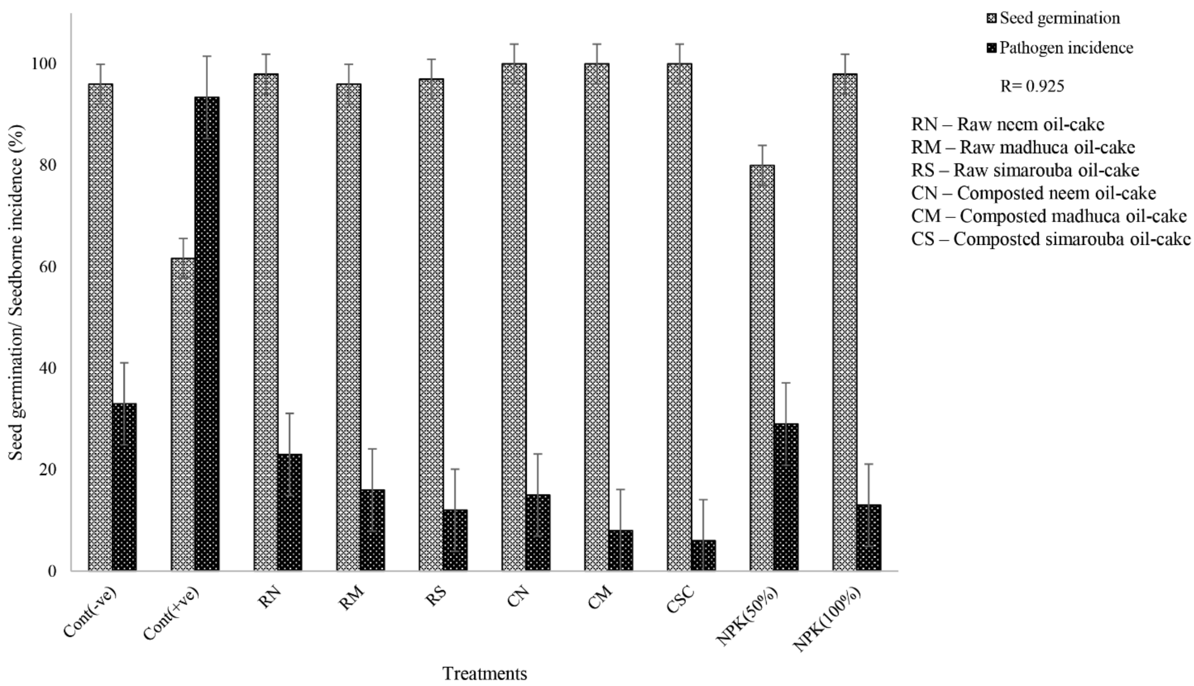

Fig. 4 Effect of soil amendment with raw or composted oil-cakes of neem, madhuca and simarouba on seed germination (%) and seedborne incidence (%) of *Alternaria tenuissima* in chilli in the field

The role of microbes in plant growth and disease management is well documented following soil amendments with organic matter (Bonanomi et al. 2010) but not with respect to oil-cakes selected in the present study. In the current study, the amendment of either the raw or composted oil-cakes to the soil stimulated a higher microbial population in the rhizosphere than in the unamended control treatments. This observation corroborated with that of the previous findings (Salem et al. 2012; Zhang et al. 2020). It was attributed to the altered microbial diversity in the root and rhizosphere after organic amendments to soil (Obermeier et al. 2020; Zhang et al. 2020). In the present study, a high density of bacterial species and phosphate solubilizers was documented at 60 DAT in the composted simarouba cake, followed by madhuca and neem cakes. Madhuca compost and raw oil-cakes attracted a low density of phosphate solubilizers when compared to composted neem and simarouba cakes. On the other hand, *Azotobacter* and *Pseudomonas* populations were high in composts in the order of madhuca>neem>simarouba as compared to other treatments. The stimulated bacterial population in the composted oil-cake treatments could be due to the readily available forms of stable mineral nutrients in the compost (Chang et al. 2007). Among the raw oil-cake amendments, neem supported high bacterial density, while the raw madhuca supported the least (Table 5). This suggested that the soil microbial composition is determined not only by the chemical components of the native soil and soil organic matter (Mendes et al. 2015) but also by the exogenously supplied organic amendments (Ling et al. 2016). The enhanced beneficial microbial population by the application of oil-cakes and their composts confirmed their potential application in agriculture (Bellini et al. 2020). Since certain raw oil-cakes contain toxic components, they might have retarded the microbial activity initially, but as the toxic compounds get degraded during composting and stabilization, the microbial population increased substantially (VasudhaUdupa et al. 2017a). Backer et al. (2018) reviewed those microbes in the rhizosphere which play key roles in nutrient acquisition and assimilation and improve soil structure by secreting and modulating extracellular molecules such as hormones, secondary metabolites and antibiotics all leading to the enhancement of plant growth.

In the present study, plant growth parameters (length, fresh and dry biomass of shoot) positively correlated with the density of beneficial microbes like *Pseudomonas,* a free nitrogen fixer and *Trichoderma* (R=0.73) but negatively correlated with the pathogen density (R= -0.12). *Pseudomonas* species are reported to promote plant growth by producing the IAA, ammonia, solubilized phosphate, siderophores and hydrogen cyanide (Pandey and Guptha 2020). *Azotobacter* species are free-living nitrogen fixers capable of converting free N_2 to available ammonia form, which is essential for plant growth (Bhattacharyya and Jha 2012). The above categories of bacteria have been studied previously for their ability to enhance plant growth (Jahanian et al. 2012). The population density of *Trichoderma* sp. was higher in composted madhuca cake than in the neem and simarouba cakes (Table 6). Among the saprophytic and beneficial fungi, *Trichoderma* spp. are rhizospheric with the ability to promote plant growth and produce organic acids to dissolve minerals and decompose nitrogen compounds into the available form (Maeda et al. 2015; Ye et al. 2020), improve nutrient uptake and produce phytohormone (Vinale et al. 2008; Contreras-Cornejo et al. 2016).

The yield and vitamin C content of chilli fruits are also positively correlated with the density of the beneficial microbes and the maximum correlation of fruit yield is with phosphate solubilizing bacteria (R=0.90). Phosphate is an essential element for plant growth and development, particularly at flowering and fruit setting stages (Walpola and Yoon 2012; Satyaprakash et al. 2017). The phosphate availability to plants occurred through the production of organic acids (Selvi et al.

Table 5 Effect of soil amendment with raw or composted oil-cakes of neem, madhuca and simarouba on the population density of bacterial species[1]

Treatments	Microbial density/Days after transplantation											
	Total bacteria			Phosphate solubilizers			Free-living N_2 fixers			*Pseudomonas* species		
	30	60	120	30	60	120	30	60	120	30	60	120
Cont. (-ve)[2]	30[3] ±2a[4]	80±1.4a	45±1.5 a	12±2.3a	28±1.6a	13±1.6a	9±1.8a	18±1.9a	12±1.5a	5±1.9a	10±1.8a	9±1.7a
Cont. (+ve)[5]	32±1.5a	82±2.1a	39±1.3a	13±2a	26±1.7a	12±1.5a	8±1.5a	15±1.9a	11±1.5a	6±1.8a	12±1.7a	9±1.8a
Raw neem oil-cake	68±1.8b	115±2.1b	65±1.9 b	25±2.4b	37±1.8a	27±1.4b	19±1.6a	31±1.5b	18±1.6a	17±1.7b	33±1.9b	25±1.9b
Comp[6]. neem oil-cake	91±1.3c	140±1.6d	109±1.5d	45±1.7c	61±1.3c	48±1.7c	25±1.7b	41±1.5c	29±1.5b	19±1.8b	34±1.5b	22±1.7b
Raw madhuca oil-cake	72 ±1.2b	109±1.7b	73±1.8 b	28±1.5b	39±1.9b	21±1.8a	15±1.6a	28±1.9a	17±1.8a	11±1.7a	23±1.6a	19±1.9a
Comp. madhuca oil-cake	115±1.3d	143±1.2d	110±1.7d	46±1.4c	53 ±2b	39±1.6b	30±1.5b	43±1.7c	34±1.5c	25±1.8c	41±1.7c	28±1.6b
Raw simarouba oil-cake	76±1.5b	129±1.3c	85±1.8c	19±1.9a	32±1.5a	28±1.7b	17±1.5a	31±1.8b	25±1.5b	16±1.8b	34±1.9b	27±1.8b
Comp.simarouba oil-cake	101±1.3d	145±1.7d	96±1.6c	52±1.5c	75±1.6 c	49±1.9c	27±1.6b	36±1.9c	23±1.6a	18±1.9b	25±1.5b	19±1.7a
NPK (50%)[7]	43±1.5a	73±1.9a	52±1.8b	15±1.8a	25±1.4a	11±1.5a	15±1.4a	21±2a	18±1.7a	7±1.3a	19±1.6a	15±1.5a
NPK (100%)[8]	80±1.3b	110±2b	82±1.6b	21±1.4b	38±1.5b	23±1.6a	20±1.6b	31±1.6b	25±1.9b	14±1.2b	34 ±1.7b	28±1.6b

[1]Experiment was conducted in completely randomized design; [2] Control without pathogen; [3]Three replicates at 10^{-6} dilution in NA, Pikovoskay's Agar, Waksman No. 77, Kings B agar media [4]Mean±SE followed by the same letter are not significantly different according to DMRT (P≤0.05); [5]Control with a pathogen; [6]Composted; [7]NPK (50:25:25) @ 50%; [8]NPK (100:50:50) @ 100%

2017; Kumar et al. 2018) and the phosphate solubilizing bacteria aid in the mineralization of organic phosphates (Khan et al. 2009; Sharma et al. 2013). Some examples of phosphate solubilizers include species of *Pseudomonas*, *Bacillus*, *Rhizobium*, *Micrococcus*, *Flavobacterium*, *Achromobacter*, *Erwinia* and *Agrobacterium* which contributed to high crop yield (Rodriguez and Fraga 1999; Satyaprakash et al. 2017). On the other hand, an increase in the density of *A. tenuissima* negatively correlated with the fruit number (R=-0.808), shoot length (R=-0.643) and dry biomass (R=-0.655) of the chilli fruits which suggested that the pathogen *A. tenuissima* directly affected the chilli yield.

Table 6 Effect of soil amendment with raw or composted oil-cakes of neem, madhuca and simarouba on the population density of fungal species[1]

Treatments	Microbial density/Days after transplantation								
	Total fungal species[2]			*Trichoderma* species			*Alternaria tenuissima*		
	30	60	120	30	60	120	30	60	120
Cont. (-ve)[3]	12±0.8 a[4]	19±1.1a	9±0.8a	4±0.9a	8±1.1a	5±0.9a	6±0.7a	3±0.9a	2±0.8a
Cont. (+ve)[5]	21±0.9 a	30±0.9b	15±0.8a	5±0.8a	9±0.9a	4±0.8a	17 ±0.8b	13±0.8b	6±0.9a
Raw neem oil-cake	25±1 b	35±1b	30±0.7b	10±1a	18±1a	12±0.7a	13±1a	7±0.9a	2±0.8a
Comp. neem oil-cake	35 ±1.1c	40±0.8b	25±0.7a	18±0.7b	28±0.6c	15±0.9b	11±1.1a	4±0.8a	1±0.8a
Raw madhuca oil-cake	20 ±1.2a	39±0.9b	21±0.8a	9±0.9a	19±0.8b	13±0.9a	10 ±0.9a	5±0.8a	3±0.7a
Comp. madhuca oil-cake	42±0.7c	48±1.1c	32±0.9b	23±0.8b	31±0.8b	19±0.8b	12 ±0.8a	5±0.8a	1±0.8a
Raw simarouba oil-cake	20±0.8a	39±1.2b	21±0.5a	9±0.7a	19±0.9b	13±1a	10±0.8a	5±0.9a	3±0.8a
Comp. simarouba oil-cake	39±0.8c	43±0.9c	27±0.7b	15±0.8b	25±0.7b	13±1.1a	11±0.8a	3±0.9a	1±0.9a
NPK (50%)[6]	24±0.9b	38±0.8b	23±0.4a	8±0.8a	15±0.6a	12±0.9a	14 ±0.7a	8±0.8a	4±0.8a
NPK (100%)[7]	38±0.8c	40±0.7b	19±0.8a	11±0.7a	19±0.8b	9±0.8a	13±0.8a	7±0.7a	3±0.7a

1Experiment was conducted in a completely randomized design; 2 Total fungi excluding A. tenuissima; 3Control without pathogen; 4Three replicates at 10-4 dilution on PDA. Means±SE followed by same letter are not significantly different according to DMRT (P≤0.05);5Control with pathogen; 6NPK (50:25:25) @ 50%; 7NPK (100:50:50) @ 100%

The present study revealed that the incidence of *A. tenuissima* was reduced with the concurrent increase in the *Trichoderma* population in both raw and composted oil-cake treatments (Table 6). The reduction in pathogen density in soil by an increase in *Trichoderma* spp. density could be attributed to the mechanisms such as secretion of cell wall degrading chitinases, glucanases, cellulase, antibiotics, and mycoparasitism (Contreras-Cornejo et al. 2016). In addition, some of the compounds such as glioviridin, viridian and gliotoxin produced by antagonists have antifungal activity (Yasmin et al. 2017; Bulgari et al. 2020) and cause a reduction in the pathogen population in the soil. The improvement in beneficial rhizosphere microbes and chilli fruit yield coupled with leaf spot disease reduction in chilli by the application of oil-cakes of neem, madhuca and simarouba and their composts have been documented so far. This study opens the way for further investigation on the use of other oil-cakes for enhancing crop production and yield beside increasing soil fertility and crop disease management.

Conclusion

The soil amendment with non-edible raw and composted oil-cakes of neem, madhuca and simarouba enhanced plant growth in both the greenhouse and field trials as well as the yield of chilli in the field. The above oil-cakes in raw or composted form were also effective in reducing the leaf spot disease incidence caused by *A. tenuissima* in chilli. The yield improvement correlated with the leaf spot disease reduction due to treatment with composted oil-cakes. The high yield of chilli fruit with enhanced vitamin C content was evident due to treatment with oil-cake composts of simarouba and neem and oil-cakes also improved soil microbial population relating to plant growth promotion, phosphate solubilization, nitrogen fixation and biological control and reduced pathogen population. The greenhouse and field experimentation demonstrated that composted non-edible oil-cakes could be used for soil amendment, to increase the plant growth and yield as well as to manage *A. tenuissima* leaf spot disease in chilli crop. The present study suggested that the composted non-edible oil-cakes, the by-product of the biodiesel industry could be used to enhance plant productivity and improve soil health by enhancing the rhizosphere microbial population.

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Compliance with ethical standards

Conflict of interest The authors declare that there are no conflicts of interest associated with this study.

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