Effect of organic manures and biofertilizers on soil enzyme activities under black pepper (Piper nigrum L.)

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Abstract
The experiment was conducted to evaluate the effects of organic manures and biofertilizers on soil enzyme activities under Black pepper (Piper nigrum L.) cultivating soils at Horticultural Research Station (TNAU), Yercaud, Salem District. During the study period, the higher available nitrogen content of 168.19 kg ha\(^{-1}\) was recorded in the treatment T\(_5\). Higher available phosphorous content of 8.35 kg ha\(^{-1}\) was recorded in the treatment T\(_2\) and higher available potassium (224.32 kg ha\(^{-1}\)) content was recorded in T\(_5\). Among all the treatments, T\(_5\) had significantly higher amount of urease, phosphatase and dehydrogenase activities. The combined application FYM, Azospirillum, Phosphobacteria and VAM have a great impact on soil nutrients and enzyme activities under Black pepper (Piper nigrum L.) cultivating soil.

Keywords: Organic manures, biofertilizers, soil enzyme, Piper nigrum L.

Introduction
Soil organic matter affects crop growth and yield, either directly by supplying nutrients or indirectly by modifying the soil physical properties, thereby improving the root environment and thus, stimulating plant growth (Avnimelech 1986)\(^{[2]}\). Crop production based on the use of organic manures rather than chemical fertilizers is assumed to be a more sustainable type of agriculture (Chang et al. 2007)\(^{[7]}\). Thimmavarayappa et al. (2000)\(^{[29]}\) indicated that highest yield of spices and total aerial dry matter were associated with the highest organic matter contents of the soils.

Soil enzymes and microbes are plays a major biochemical functions in the overall process of organic matter decomposition in the soil ecosystem (Sinsabaugh & Moorhead 1994)\(^{[22]}\). The activities of an enzyme in soil systems differ in amounts mainly due to the fact that each soil type has different amounts of organic matter content, an intensity of the biological processes and composition and activity of living organisms (Sudhakaran et al. 2018)\(^{[28]}\). Soil enzymes are very sensitive to the changes in soil environment due to tillage, amendments, crop rotation and agricultural management practices. Floch et al., (2009)\(^{[10]}\) & Sudhakaran et al. 2019 were also reported that the soil enzyme activities are higher in organically managed farming when compared to the conventional and integrated managed farming. Black pepper (Piper nigrum L.) is the major spice produced in India. Kerala is the largest producer of black pepper in India with a production of about 25,000 tons, contributing 51% of the share in the overall production and occupying 95% area under pepper cultivation (2007-08) followed by Karnataka (37%) and Tamil Nadu (6%) (Ravindr 2000)\(^{[18]}\). In Tamil Nadu, it is mainly grown in scattered localities on the eastern slopes of the Western Ghats. It is grown as an intercrop in coffee, tea and arecanut plantations especially in Pulney and Shevaroy hills where the vines are trained on shade trees (Balasubramanian 2016)\(^{[4]}\).

Since black pepper is usually grown on large scale in virgin soils, it is believed by most of the cultivators that special manuring is not necessary for black pepper (Sayeed,1963)\(^{[21]}\). But continuous cropping over a period of years brought about progressive decline in yield, mainly due to depletion of soil fertility. It is therefore absolutely necessary that the crop has to be systematically and judiciously manured to maintain high yield (Kandianman 2000)\(^{[12]}\).
Nowadays, a lot of attention is being given to biofertilizer application in horticultural crops to reduce the dose of inorganic fertilizers and also reduce the production cost. With the above background, present investigation was proposed with the aim of organic manures and biofertilizers effects on soil enzyme activities under Black pepper (Piper nigrum L.) cultivating soils.

Materials and Methods
Study area and Experimental details
The present investigation on Standardization of Organic Practices in Black Pepper Cv., Panniyur- 1 was carried out at the Horticultural Research Station, Tamil Nadu Agricultural University, Yercaud, Salem District in Tamil Nadu in 2008-09. The Research Station is situated at an altitude of 1500 m above MSL between 1° 04’ to 11° 05’ North latitude and 78° 05” to 78° 23” East longitude. The average annual rainfall of Yercaud is 1500 mm and the mean maximum and minimum temperature are 21.0 to 32.2 °C and 11.0 to 18 °C, respectively. The mean relative humidity during the period was 58 to 92 per cent. The soil of the experimental plot is laterite in texture with 0.5 to 1.5 m depth. The pH of experimental site is 4.5 to 6.0.

The experiment was laid out in Randomized Block design with consist of six treatments viz, T1 - FYM 10 kg + 5 kg Coir Compost + 50 g Phosphobacteria + 50 g Azospirillium, T2 - FYM 10 kg + 1 kg Vermi Compost + 50 g Phosphobacteria, T3 - FYM 10 kg + 1 kg Neem Cake + 50 g Phosphobacteria + 50 g Azospirillium, T4 - FYM 10 kg + 50 g Azospirillium + 50 g Phosphobacteria, T5 - FYM 10 kg + 50 g Azospirillium + 50 g Phosphobacteria + 200 g VAM and T6 - 100 g of N + 40 g of P₂O₅ + 140 g of K₂O (Recommended dose for Package of Practices) - Control.

Sample collection and soil parameter analysis
The soil samples were collected during three different stages viz, before flushing (S1), during peak flowering (S2), after harvest (S3). Soil samples from 0 to 25 cm depth were collected from four points in the basin, 15 cm away from the vine and composted to give a sample. Soil samples from all the treatment vines were collected separately. Collected soil samples were air dried, powdered gently and passed through 2 mm sieve. The samples were kept in labelled polythene bags for further analysis.

Soil pH (1:2 soil-water suspension) was determined by potentiometry (Bashour & Sayegh 2007) [5]. Available nitrogen was determined by Alkaline permanganate method (Bashour & Sayegh 2007) [5]. Available phosphorus was determined by modified Olsen method (Olsen & Sommers 1982) [16]. Available potassium was determined by flame photometry (Stanford & English 1949) [23]. Urease activity (mg NH₄-N g⁻²h⁻¹) was measured by ammonia released after the incubation of sample with urea solution for 2 h at 37 °C (Tabatabai & Bremner 1972) [28]. Phosphatase activity (µ moles PNP released g⁻¹ min⁻¹) was estimated by paranitrophenol released after the incubation of soil with p-nitrophenyl phosphate for 1 h at 37 °C (Tabatabai & Bremner 1969) [27]. Dehydrogenase (DHG) activity (Δ in OD at 485 nm) was estimated following by Klein et al. (1971) [13].

Results and Discussion
Effect of organic manures and biofertilizers on soil nutrient content
The initial nitrogen content in the soil was 156.18 kg ha⁻¹. At flowering stage, the nitrogen content ranged from 157.72 kg ha⁻¹ (T₆) to 168.19 kg ha⁻¹ (T₃) (Table 1). At harvesting stage the range was 135.11 kg ha⁻¹ (T₆) to 153.63 kg ha⁻¹ (T₃). Higher nitrogen content of 168.19 kg ha⁻¹ was recorded in the treatment T₃ at flowering stage and 153.63 kg ha⁻¹ was recorded in the treatment T₃ at flowering stage. T₆ treatment recorded significantly lower levels of nitrogen in both the stages (157.72 and 153.11 kg ha⁻¹) at both the stages and the treatments T₃ were on par with them (Table 1).

From the initial 7.56 kg ha⁻¹ the soil phosphorus content was increased to 7.71 kg ha⁻¹ in T₆ and 8.35 kg ha⁻¹ in T₂ at flowering stage (Table 13). The range was 6.90 kg ha⁻¹ (T₆) to 7.44 kg ha⁻¹ (T₂) at harvesting stage. Higher phosphorus content of 8.35 kg ha⁻¹ was recorded in the treatment T₂ at flowering stage and 7.44 was recorded in the treatment T₃ at harvesting stage. T₆ treatment recorded significantly lower level of phosphorous (7.71 and 6.90 kg ha⁻¹) at both the stages and the treatments T₃ was on par with them (Table 1).

The combined application of FYM, Azospirillum, Phosphobacteria and VAM showed greater potassium content (224.32 kg ha⁻¹) at flowering stage and (206.09 per cent) at harvesting stage. The lower potassium content was observed in T₆ (214.20 and 192.11 per cent) at both the stages (Table 1).

Fallowyard manure could supply 5.0 Kg N ton⁻¹ (Jaipaul, 2011) [11] and Neem Cake contains 5.6 % of N. So the increase in the contents of total nitrogen in the soil might be attributed to the better availability of nitrogen coupled with related nitrification process enabling presence of nitrogen for a longer period in the soil. This is in corroboration with previous findings of Thimmavarayappan et al. (2000) [29], Azospirillum would have enhanced the soil available nitrogen because of its non-symbiotic nitrogen fixation. This is in confirmation with earlier observation of Lai (2008) [15]. Similarly, Azospirillum might be responsible for the promotion of availability of ion by scavenging the nutrients. The present study is in consonance with the findings of Sathiyavathi and Ramanathan (2001) [20]. Organic nitrogen and P₂O₅ availability in the soil increased with the application of FYM, Vermicompost, Azospirillum and Phosphobacteria due to the increase of decomposition of products of organic matter (Sudhakaran et al. 2018) [25]. Moreover, vermicompost possesses high ‘P’ content. Application of vermicompost in this treatment could be responsible for the high soil ‘P’ content. The inoculation of phosphobacteria resulted in the increased availability of phosphorous, since these bacteria helps to degrade the complex forms of phosphate into more soluble and simple forms of phosphorous. The present investigation is in agreement with that of Basu et al. (2006) [6], Balakrishnan et al. (2007) [3] and Chen & Liu (2008) [8].

The highest potassium content in soil was noticed under the application of FYM along with Azospirillum, Phosphobacteria and VAM. This could be due to the application of FYM, which helps to convert into soil humus substances and mobilization of potassium due to the exchange reaction with soil particles. This is in corroboration with the previous works of Sandhya et al. (2013) [19]. Azospirillum may also be ascribed to enhance the availability of potassium.
Effect of organic manures and biofertilizers on Soil enzyme activities

Significant difference was observed on urease enzyme activity, T₃ registered significantly the higher urease activity of (135.8, 146.3 and 129.2 µg NH₄-N released g⁻¹ h⁻¹) at initial stage, flowering and harvesting stage respectively. This was followed by T₄ which recorded (131.1, 140.9 and 125.4 µg NH₄-N released g⁻¹ h⁻¹) at initial stage, flowering and harvesting stage respectively (Table 2). Soil urease mainly originate from plant roots and microorganisms, which is influenced by many factors. These include organic matter content of the soil, cropping pattern, soil depth and soil amendments (Acosta-Martinez et al. 2003) [1]. Sudhakaran et al. (2018) [20] also reported that urease activity is direct indicator of nitrogen availability in agricultural soils. Among all the treatments FYM + Azospirillum + Phosphobacteria + VAM (T₄) showed higher phosphatase activity (0.14, 0.14 and 0.12 µ moles PNP released g⁻¹ min⁻¹) than absolute control (T₀) and other treatments and on par with FYM + Azospirillum + Phosphobacteria + Neem cake (T₅) was given in table 3. Phosphatase enzyme secreted from plant roots and soil microbes to enhance the solubilization and remineralization of PO₄₃⁻. Phosphatase plays an important role in converting of organic phosphorus to plant liable phosphorus (Chinnadurai et al. 2014).

Significant variation was recorded among the six treatments studied for dehydrogenase activity (Table 2). It varied from 0.11 to 0.26 at initial stage, 0.19 to 0.32 at flowering stage and at harvesting stage (0.11 to 0.13). The dehydrogenase activity was high in T₃ (0.26, 0.32 and 0.13) in three stages and it was on par with T₄. The dehydrogenase activity was low in T₅. This might be due to higher organic manure application which would have favored more microbial populations ultimately more enzyme activity. Similar strong relationship between organic manure and enzyme activities have been reported by Krishnakumar et al. (2005) [14] and Chang et al. (2007) [13].

Table 1: Effect of organic practices on soil nutrients

| Treatments | Available N (kg ha⁻¹) | Available P (kg ha⁻¹) | Available K (kg ha⁻¹) |
|------------|----------------------|-----------------------|----------------------|
|            | IS       | FS   | HS     | IS       | FS   | HS     | IS       | FS   | HS     |
| T₁         | 156.18   | 165.52 | 152.11 | 7.56     | 8.12  | 7.35   | 210.6    | 215.54 | 201.22 |
| T₂         | 156.18   | 168.11 | 144.35 | 7.56     | 8.35  | 7.44   | 210.6    | 219.65 | 203.14 |
| T₃         | 156.18   | 168.19 | 153.63 | 7.56     | 8.21  | 7.28   | 210.6    | 221.34 | 205.48 |
| T₄         | 156.18   | 158.34 | 135.82 | 7.56     | 7.89  | 7.09   | 210.6    | 217.36 | 202.88 |
| T₅         | 156.18   | 161.67 | 145.86 | 7.56     | 8.09  | 7.10   | 210.6    | 224.32 | 206.09 |
| T₆         | 156.18   | 157.72 | 135.11 | 7.56     | 7.71  | 6.90   | 210.6    | 214.20 | 192.11 |
| SEd        | 2.3482   | 2.0826 |        | 0.1151   | 0.1039|        | 3.1457   | 2.9018 |        |
| CD (P=0.05)| 5.0052   | 4.4390 |        | 0.2454   | 0.2215|        | 6.7050   | 6.1851 |        |

IS: Initial Stage, FS: Flowering Stage and HS: Harvesting Stage

Table 2: Effect of organic practices on soil enzyme activities

| Treatments | Urease (µg NH₄-N released g⁻¹ h⁻¹) | Phosphatase (µ moles PNP released g⁻¹ min⁻¹) | Dehydrogenase (A in OD at 485 nm) |
|------------|-----------------------------------|---------------------------------------------|----------------------------------|
|            | IS       | FS   | HS       | IS   | FS   | HS   | IS   | FS   | HS   |
| T₁         | 120.00   | 131.6 | 110.9    | 0.13 | 0.21 | 0.10 | 0.18 | 0.25 | 0.18 |
| T₂         | 125.70   | 137.1 | 119.0    | 0.18 | 0.29 | 0.15 | 0.15 | 0.23 | 0.15 |
| T₃         | 131.1    | 140.9 | 125.4    | 0.21 | 0.32 | 0.19 | 0.23 | 0.29 | 0.22 |
| T₄         | 121.0    | 129.4 | 112.0    | 0.16 | 0.24 | 0.13 | 0.14 | 0.21 | 0.13 |
| T₅         | 135.8    | 146.3 | 129.2    | 0.25 | 0.39 | 0.20 | 0.26 | 0.32 | 0.25 |
| T₆         | 115.3    | 125.0 | 102.3    | 0.11 | 0.25 | 0.08 | 0.11 | 0.19 | 0.11 |
| SEd        | 1.7991   | 1.9470 | 1.6818  | 0.0042 | 0.0063 | 0.0033 | 0.0024 | 0.0038 | 0.0014 |
| CD (P=0.05)| 3.8346   | 4.1500 | 3.5847  | 0.0090 | 0.0134 | 0.0071 | 0.0050 | 0.0081 | 0.0031 |

IS: Initial Stage, FS: Flowering Stage and HS: Harvesting Stage

Conclusion

From the above study, it is concluded that the combined application FYM, Azospirillum, Phosphobacteria and VAM have a great impact on soil nutrients and soil enzyme activities under Black pepper (Piper nigrum L.) cultivating soils. In general, the available N, P and K in the soil, declined from initial stage to harvesting stage indicating the higher uptake of nutrients from the soil as the stages advances. Soil enzyme activity and biomass are influenced the higher biomass and soil enzymes in the treatment FYM, Azospirillum, Phosphobacteria and VAM (T₃).

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