The Pharma Innovation Journal 2021; 10(10): 2128-2133

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2021; 10(10): 2128-2133

www.thepharmajournal.com
Received: 14-08-2021
Accepted: 27-09-2021

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Conjunctive effect of organic manures and pyroligneous acid on soil enzyme activity in sodic soil

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Abstract
A pot experiment was conducted in Regional Laboratory at Anbil Dharmalingam Agricultural College and Research Institute, Tiruchirapalli to study the effect of organic manures and pyroligneous acid on soil enzymes activity under okra cultivation in sodic soil. The result of the study was the improvement in soil enzymes in all plots over the initial value up to 60 DAS which declined thereafter. On 60 DAS, highest urease (11.63 µg NH_4^+ released g^{-1} hr^{-1}), dehydrogenase (40.03 µg of TPF g^{-1}) and alkaline phosphatase (42.00 µg of PNPP g^{-1}) activity was found in the treatment T_{10} (STCR + PA @ 1:300 dilution + 2% vermicompost). Increase in the soil enzyme activity is a good indicator of soil condition for crop growth. The Study revealed that use of organic manures along with PA increased soil enzymes viz., urease, dehydrogenase and alkaline phosphatase activity when compare to sole application PA (T_2), FYM (T_5) and vermicompost (T_7).

Keywords: Pyroligneous acid, urease, dehydrogenase and alkaline phosphatase

Introduction
Vegetables play an important role in human diet as they are the chief source of carbohydrates, minerals, vitamins, proteins and dietary fibers. Okra (*Abelmoschus esculentus*) called the "Queen of vegetables" belongs to the family Malvaceae and it is locally known as Bhendi and Lady's finger. Globally, India ranks second in vegetable production accounting for 6,371 MT and occupies an area of 519 Mha (NHB, 2019). In Tamil Nadu, okra is one of the main vegetable crops grown in the district of Dharmapuri, Salem, Dindugal, Thiruvallur and Thiruvanamalai and occupies an area of 11,824 ha, with a production of 1,24,887 MT and productivity of 10.56 MT/ ha (Department of horticulture and plantation crops, Government of Tamil Nadu, 2017-2018). The production of okra is mainly affected by the use of sub-optimal and inappropriate organic manure doses. The reduced crop yield is attributed to low fertilizer use efficiency due to soil pH and nutrient imbalance (Obi and Ebo, 1995) [16]. Besides, the constant escalation in cost of inorganic fertilizers has lead to the use of locally available organic material (both manures as well as wastes) which has resulted in improved soil physical properties, use efficiency of inorganic fertilizer and increased yield (Somani and Totawat, 1996) [22]. Excessive salts in soil have affected agricultural output which account for an estimated 20% of the world's irrigated acreage (Qadir *et al.*, 2006) [20]. Sodic soils are defined by the presence of excessive sodium ions (Na^+) in soil solution and in the exchange phase up to levels that can adversely affect the physical, chemical, and biological properties of soils as a category of salt-affected soils (Liang *et al.*, 2003) [12]. In India, nearly 6.73 million ha are affected by salt, while 3.77 million hectares of land is affected by sodicity. Sodic soil covers around 3.5 lakh hectares in Tamil Nadu alone, of which Trichy accounts for 5800 hectares. The soil physical properties are significantly affected by high sodicity characterized by ESP >15 and pH >9. They contain sufficient exchangeable sodium to inhibit the growth of most crop plants. Excess exchangeable salt has significant effects on the physical and nutritional qualities of the soil, resulting in a huge or complete loss in crop growth. Sodic soil reclamation includes the use of chemical ameliorants (gypsum and calcium chloride) as well as organic amendments such as farmyard manure, municipal solid waste and green manures (Hanay *et al.*, 2004; Tejada *et al.*, 2006) [8, 26]. Use of organic amendments in sodic soils could be a better management alternative than chemical fertilizers, with the additional benefit of increased physical properties and soil biological characteristics. According to Li & Keren (2009) [11], using organic amendments increased the CO_2 partial pressure in the soil profile, thereby lowering the pH in soil solution which resulted in an increase in the dissolution of native

mineral $CaCO_3$ and reduction in soil sodicity. Pyroligneous acid (PA) or Wood vinegar (WV) is an acidic reddish-brown aqueous liquid. It is produced by clarifying the liquid product of the carbonization process of wood residues from wood processing industry, tree branches and other biomaterial such as bamboo, corn cobs, crop straw, fruit shell which are used as raw material (Wei *et al*., 2010) [21]. PA is known for its multi - dimensional use as soil or foliar fertilizer and plant growth promoter or inhibitor, insect repellent, odor remover and wood preservative. Soil enzyme activity is a key indicator of soil health. These enzymes play biochemical functions through their catalytic action. They are involved in the organic matter decomposition (e.g., hydrolase, glucosidase), nutrient transformation (e.g., amidase, urease, phosphatase, sulphatase) and nutrient cycle which are related to soil fertility (Dick, 1994) [6]. Nearly 500 enzymes are reported to play important roles in the C, N, P, and S cycles in soil. Pyroligneous acid application at different concentration in sandy soil was observed that effect on microbial biomass and enzyme activity (β-Glucosidase, alkaline phosphatase and dehydrogenase) (Du *et al*., 2016) [7]. The objective of this study was to determine the effect of organic manures and pyroligneous acid applied either alone or in combination on soil biological activity and soil namely urease, dehydrogenase and alkaline phosphatase.

Materials and Methods

Collection and analysis of soil samples

A composite surface soil sample (0-15 cm) was collected from Anbil Dharmalingam Agricultural College and Research Institute farm, Tiruchirapalli. The collected soil samples were air dried, processed and passed through 2 mm sieve for pot culture experiments. The soil samples were analyzed for various physio- chemical properties. The fresh soil samples were analyzed for enzyme activity, namely urease, dehydrogenase and alkaline phosphatase. The bulk density, particle density and porosity was determined by the cylinder method (Piper, 1966). Soil textural analysis was determined by International pipette method (Piper, 1966). The pH and EC (1:2.5) of soils were determined by a pH meter and a conductivity meter (Jackson, 1973) [10]. The organic carbon content of the soil was determined by wet chromic acid digestion method (Walkley and black, 1935). The CEC of soil was estimated by sodium acetate method (Bower *et al.,* 1952) [2]. The soil samples were analyzed for available N by the alkaline permanganate method (Subbiah and Asija 1956), available P (Olsen- P) by 0.5M sodium bicarbonate ($NaHCO_3$) extraction (Olsen *et al.,* 1954) [17], available K (NH_4OAc) by 1N neutral NH_4OAc extraction on flame photometer (Jackson, 1973) [10]. The method employed for assay of urease was developed by Tabatabai and Bremmer (1972) [25]. The soil was added with 0.2 ml of toluene, 9ml of Tris Hydroxymethyl Amino methane (THAM-0.05 M, pH 9.0). Then 1 ml of 0.2 M urea solution was added and incubated for 2 hours at 37^0 C. Dehydrogenase enzyme activity was determined by the reduction of triphenyl tetrazolium chloride (TTC) to triphenyl formazan (TPF) as described by (Casida Jr *et al*., 1964) [4]. Alkaline phosphates was developed by (Tabatabai and Bremmer 1969) [24]. One gram of the soil in 100ml Erlenmeyer flask was added with 0.2ml of toluene and 4ml of modified universal buffer (pH 11.0) was added, followed by 1 ml of 0.05 M *p*-nitrophenyl phosphate (pH 11.0) and kept in incubator for one hour. The intensity of the yellow colour was measured immediately in an UV- spectrophotometer at 420 nm. The microbial population of bacteria, fungi and actinomycetes were estimated by the serial dilution pour plate technique (Chhonkar *et al*., 2007) [5].

Pot experiment

The pot culture experiment was conducted at Regional Laboratory at Anbil Dharmalingam Agricultural College and Research Institute, Tiruchirapalli, Tamil Nadu. Nine kilograms of soil was weighed and transferred to each plastic pot. At the time of pot filling, broken pieces of stones were placed at the bottom with a hole to allow free drainage. Calculated quantities of recommended dose of fertilizer (RDF) viz., N, P_2O_5 and K_2O were applied as basal dose to the treatments T_3, T_5, T_6, T_8 and T_9 pots while calculated quantities of soil test crop response (STCR) recommendation of N, P_2O_5 and K_2O were applied as basal dose to T_4, T_7 and T_{10} pots as per TNAU CPG and mixed thoroughly. FYM at the rate of 2% (180g/pot) was applied to the treatments T_5, T_6 and T_7 while 2% vermicompost (180g/pot) was applied to treatments T_8, T_9 and T_{10}, respectively. The soil was drenched (SD) with 1.5 liters of PA @ 1:300 dilution (i.e. 1ml of PA+ 300ml of water) which was applied to treatment T_2, T_3, T_4, T_6, T_7, T_9 and T_{10} one day prior to sowing. Okra seeds were procured from Tamilnadu Agricultural University, Coimbatore- TNAU (Bhendi hybrid CO 4). Pyroligneous acid extracted from coconut shell was used in the study. Farmyard manure and vermicompost were procured from Anbil Dharmalingam Agricultural College and Research Institute farm, Tiruchirapalli. The collected organic manures were air dried, processed and passed through 2 mm sieve and used for pot culture experiments. The fresh soil samples were collected for enzyme analysis at of 30, 60, 90 DAS and at post-harvest stage. The experiment was set up in a completely randomized design with ten treatments and three replications. The treatments comprised of T_1 - Absolute control, T_2 - PA @ 1:300 dilution as SD, T_3 - RDF + PA @ 1:300 dilution as SD, T_4 - STCR + PA @ 1:300 dilution as SD, T_5 - RDF + 2% FYM, T_6 - T_3 + 2% FYM, T_7 – T_4 + 2% FYM, T_8 - RDF + 2% Vermicompost, T_9 - T_3 + 2% Vermicompost, T_{10} – T_4 + 2% Vermicompost.

Result and Discussion

The initial composite soil sample was analyzed for physio-chemical characteristics and results are presented in Table 1.The soil pH was sodic with a fertility status of low available nitrogen, medium in available phosphorous and high in available potassium. The soil organic carbon was high. The enzyme activity of initial soil samples were analyzed and was found to have the urease enzyme activity of 1.6 µg NH_4^+ released g^{-1} hr^{-1}, dehydrogenase enzyme activity of 5.6 µg of TPF g^{-1} of soil and alkaline phosphatase activity of 2.4 µg of PNPP g^{-1}. The combined effect of organic manure and pyroligneous acid in the soil may have a significant effect on enzyme activity which are directly related to soil health and fertility. Urease, dehydrogenase and phosphatase are the most significant soil enzymes.

Soil enzyme activity

All the three enzyme urease, dehydrogenase and alkaline phosphatase were increased up to 60 DAS and declined thereafter days. The STCR recommended combined with organic fertilizer and pyroligneous acid @ 1:300 dilution recorded highest value in all enzymes.

Urease activity: Biological hydrolysis of urea requires the enzyme urease, which is abundant in soils. Urease activity is greatest in the rhizosphere where microbial activity is high. In this study the urease activity ranged from 2.55 to 11.91 µg NH_4^+ released g^{-1} hr^{-1} during the crop growth period of okra irrespective of the treatments and stage of sampling. The addition of vermicompost @ 2% with or without PA @ 1:300 dilution in combination with inorganic fertilizer applied either as RDF / STCR recommendation recorded higher urease activity at all stages of sampling when compared to the addition of organic manure as FYM @ 2%. The value ranged from 5.12- 7.30 µg NH_4^+ released g^{-1} hr^{-1} at 30 DAS, 9.93 to 11.91 µg NH_4^+ released g^{-1} hr^{-1} at 60 DAS, 9.16 to 10.75 µg NH_4^+ released g^{-1} hr^{-1} at 90 DAS and 6.87 to 8.40 µg NH_4^+ released g^{-1} hr^{-1} at harvest, respectively. The higher urease activity (4.31, 8.26, 7.67 and 6.71 µg NH_4^+ released g^{-1} hr^{-1} at 30, 60, 90 DAS and harvest stages, respectively) in fig 1. revealed the beneficial effect of PA @1:300 dilution in combination with inorganic fertilizers applied as RDF (T_3) in the absence of FYM @ 2% compared to T_5 (RDF + 2% FYM). The absolute control recorded lowest urease activity at all stages of crop growth. However, the application of PA alone @1:300 dilution (T_2) recorded higher values then absolute control (T_1) emphasizing the over-riding influence of the pyroligneous acid addition. The addition of organic manures either as FYM or vermicompost recorded higher urease enzyme activity when compared to absolute control and soil drenching with PA. Urease activity increased with the size of the soil microbial population and with organic matter content. (Tisdale and Nelson, 1995) [27]. Large numbers of bacteria, fungi and actinomycetes in soils possess the enzyme urease. This was clearly evident from the data on the microbial population recorded in the study (Table 2, 3 & 4). The bacteria, fungi and actinomycetes population in the treatments receiving organic matter addition either as FYM or vermicompost or PA as soil drench along with inorganic fertilizers recorded higher counts. The treatment which did not receive organic matter addition either as FYM / vermicompost / PA as soil drench (absolute control T_1) recorded the lowest microbial population count which was reflected in the lowest urease activity in the soil. The highest urease enzyme activity correlated with highest microbial population. Application of organic manures to soils could increase soil microbial biomass production (Diepeningen *et al.*, 2006) [28] resulting in enhanced nutrient transformation and uptake in plants through increased soil enzymatic activities. Heidari *et al.*, (2016) [9] also observed a similar increase in the activity of soil urease enzyme due to the addition of organic manures. The soil urease enzyme is an indicator of soil biological activity due to the addition of organic manures *viz*., FYM and vermicompost with / without soil drenching of PA. Similarly, enhanced urease activity in soils amended with sewage sludge, chicken manure and horse manure were reported by Antonious (2016) [1].

Table 1: Physio chemical properties of initial soil sample

Soil properties	
Bulk density	1.42 Mgm^{-3}
Particle density	2.50 Mgm^{-3}
Porosity	44%
Texture	Sandy clay loam
Sand	61.5%
Silt	15%
Clay	22.5%
pH	8.8
EC	0.37 dS m^{-1},
Cation exchange capacity	23 C mol (p^+)/ kg
Organic carbon	0.73%
Available nitrogen	219.52 kg/ha
Available phosphorous	20.16 kg/ha
Available potassium	350 kg/ha

Table 2: Combined effect of organic manures and soil drenching with PA on soil microbial population of bacteria (X 10^{-6} CFU g^{-1})

Treatments		30 DAS	60 DAS	90 DAS	Post-harvest
T_1	Absolute control	15.18	35.26	26.46	19.59
T_2	PA @ 1:300 dilution as SD	16.30	37.15	28.66	20.84
T_3	RDF + PA @ 1:300 dilution as SD	19.49	41.14	33.50	24.49
T_4	STCR + PA @ 1:300 dilution as SD	24.00	46.04	37.75	29.76
T_5	RDF + 2% FYM	18.26	39.44	31.58	22.56
T_6	T_3 + 2% FYM	21.20	42.28	34.73	25.18
T_7	T_4 + 2% FYM	25.56	47.47	39.55	31.36
T_8	RDF + 2% Vermicompost	22.20	44.73	36.20	27.12
T_9	T_3 + 2% Vermicompost	27.05	48.35	41.73	33.42
T_{10}	T_4 + 2% Vermicompost	29.19	50.17	44.35	35.76
SED		0.35	0.25	0.30	0.23
CD (P = 0.05)		0.72	0.52	0.63	0.49

SD – Soil drenching

Table 3: Combined effect of organic manures and soil drenching with PA on soil microbial population of fungi (X 10^{-3} CFU g^{-1})

Treatments		30 DAS	60 DAS	90 DAS	Post-harvest
T_1	Absolute control	10.70	23.44	15.15	13.05
T_2	PA @ 1:300 dilution	12.04	24.55	16.89	14.22
T_3	RDF + PA @ 1:300 dilution	15.91	27.48	20.15	17.65
T_4	STCR + PA @ 1:300 dilution	20.73	31.38	25.47	22.82
T_5	RDF + 2% FYM	13.49	25.48	18.97	15.01
T_6	T_3 + 2% FYM	17.16	28.57	21.69	19.55
T_7	T_4 + 2% FYM	21.57	32.94	27.38	24.24
T_8	RDF + 2% Vermicompost	18.89	30.12	23.98	21.35
T_9	T_3 + 2% Vermicompost	22.93	34.94	28.83	25.76
T_{10}	T_4 + 2% Vermicompost	24.09	36.25	30.54	27.50
SED		0.43	0.30	0.24	0.63
CD (P = 0.05)		0.89	0.63	0.57	0.86

SD – Soil drenching

Table 4: Combined effect of organic manures and soil drenching with PA on soil microbial actinomycetes (X 10^{-2} CFU g^{-1})

Treatments		30 DAS	60 DAS	90 DAS	Post-harvest
T_1	Absolute control	34.20	49.17	40.37	36.19
T_2	PA @ 1:300 dilution	36.20	52.06	41.11	39.43
T_3	RDF + PA @ 1:300 dilution	41.70	55.22	44.25	42.48
T_4	STCR + PA @ 1:300 dilution	45.35	60.27	55.82	52.10
T_5	RDF + 2% FYM	38.91	53.62	42.15	40.43
T_6	T_3 + 2% FYM	42.36	56.90	53.07	47.47
T_7	T_4 + 2% FYM	48.56	61.64	57.71	54.40
T_8	RDF + 2% Vermicompost	44.37	58.84	50.75	49.70
T_9	T_3 + 2% Vermicompost	51.53	64.69	59.49	56.30
T_{10}	T_4 + 2% Vermicompost	53.21	66.48	61.61	57.45
SED		0.27	0.30	0.33	0.53
CD (P = 0.05)		0.57	0.63	0.70	1.10

SD – Soil drenching

Dehydrogenase activity

The activity of the dehydrogenase enzyme is mainly employed as a measure of biological activity in soils (Burns 1982) [3]. This was revealed from the data obtained in the study. The highest microbial population for bacteria, fungi and actinomycetes was recorded with addition of inorganic fertilizer either as FYM / STCR recommendation in combination with organic manures either as FYM / vermicompost with or without addition of PA as soil drench. The highest microbial population for bacteria, fungi and actinomycetes were recorded in T_{10} (Table 2, 3, 4). Soil dehydrogenase activity measured in terms of formation of formazan from triphenyl tetrazolium chloride was also chosen as good index of microbial activity in soil. The soil dehydrogenase system is due to rather wide group of soil enzymes which transfer electrons to available acceptors. In the present study, dehydrogenase activity ranged from 6.54 to 40.03 µg of TPF g^{-1} during the crop growth period of okra irrespective of the treatments and stage of sampling. The addition of vermicompost @ 2% with or without PA @ 1:300 dilution in combination with inorganic fertilizer applied either as RDF / STCR recommendation (T_8, T_9, T_{10}) recorded higher dehydrogenase activity (Fig 2) at all stages of sampling when compared to the addition of organic manure as FYM @ 2%. The value ranged from 17.38 to 25.4 µg of TPF g^{-1} at 30 DAS, 28.83 to 40.03 µg of TPF g^{-1} at 60 DAS, 25.33 to 25.79 µg of TPF g^{-1} at 90 DAS and 2.87 to 25.79 µg of TPF g^{-1} at harvest, respectively. The application of STCR based inorganic fertilizers in combination with organic manures along with soil drenching of PA (T_{10}) recorded higher dehydrogenase activity when compared to the same combination but with RDF as inorganic fertilizer dose (T_9). This highlighted the fact that soil test-based application of inorganic fertilizer was more crucial in improving the microbial diversity and soil dehydrogenase activity than RDF indicating that an excess of inorganic fertilizer addition to soil was detrimental to soil biological activity. Similar enhanced dehydrogenase activity was reported by Kumar *et al.*, (2017) on application of 50% recommended dose of NPK + vermicompost (@ 2 t / ha) in soil. This might be due to increased microbial activity in the same treatment. Incorporation of organic manures and PA may have served as a potential source microbial diversity and activities of microorganisms accompanied by dehydrogenase activity (Nath *et al.*, 2015) [15]. The data also revealed the beneficial effect of PA @1:300 dilution in combination with inorganic fertilizer applied as RDF (T_3) than RDF in combination with FYM @ 2% (T_5). The absolute control recorded lowest dehydrogenase activity at all stages of crop growth. However, the application of PA alone @1:300 dilution (T_2) recorded higher values then absolute control (T_1) which could have contributed to improved population of soil microflora, and this was reflected in the concomitant increase in the soil enzymes as well in all the treatments that received PA as soil drench. The application of different organic manures not only increase the availability of organic matter reservoirs and nutrients in soil but also promote the biodiversity of soil microbial communities. This would have further enhanced the overall enzyme activity, nutrient transformation particularly N, P and S and nutrient cycle in soil. The data revealed positive correlation with soil microbial population and enzyme activity.

Alkaline phosphatase activity

Phosphatases play a main role in soil P mineralization (Nannipieri *et al.*, 2011) [13] and it converts organic phosphorus to inorganic phosphorous through mineralizing phosphate in soil (Omar and Abdel Sater, 2001) [18]. Alkaline phosphatase activity ranged from 5.99 to 42.00 µg of PNPP g^{-1} during the crop growth period of okra irrespective of the treatments and stage of sampling. The addition of vermicompost @ 2% with or without PA @ 1:300 dilution in combination with inorganic fertilizer applied either as RDF / STCR recommendation recorded higher alkaline phosphatase activity at all stages of sampling when compared to the addition of organic manure as FYM @ 2%. The value ranged from 16.93 to 25.06 µg of PNPP g^{-1} at 30 DAS, 29.54 to 42.00 µg of PNPP g^{-1} at 60 DAS, 24.19 to 30.72 µg of PNPP g^{-1} at 90 DAS and 18.33 to 28.03 µg of PNPP g^{-1} at harvest, respectively. The treatment receiving PA @1:300 dilution in combination with inorganic fertilizer applied as RDF (T_3) recorded higher alkaline phosphatase activity (13.01, 21.56, 15.90 and 3.34 µg of PNPP g^{-1} at 30, 60, 90 DAS and harvest) than the combination of RDF + 2% FYM (T_5) given in Fig 3. This revealed the beneficial effect of pyroligneous acid over the FYM on the alkaline phosphatase activity. The absolute control recorded lowest dehydrogenase activity at all stages of crop growth. However, the application of PA alone @1:300 dilution (T_2) recorded higher values then absolute control (T_1) indicating the positive influence of the pyroligneous acid in enhancing the biological activity in soil. Similar increase in alkaline phosphatase enzyme activity was reported by Du *et al.* (2016) [7] when pyroligneous acid was applied at different concentrations to a sandy soil.

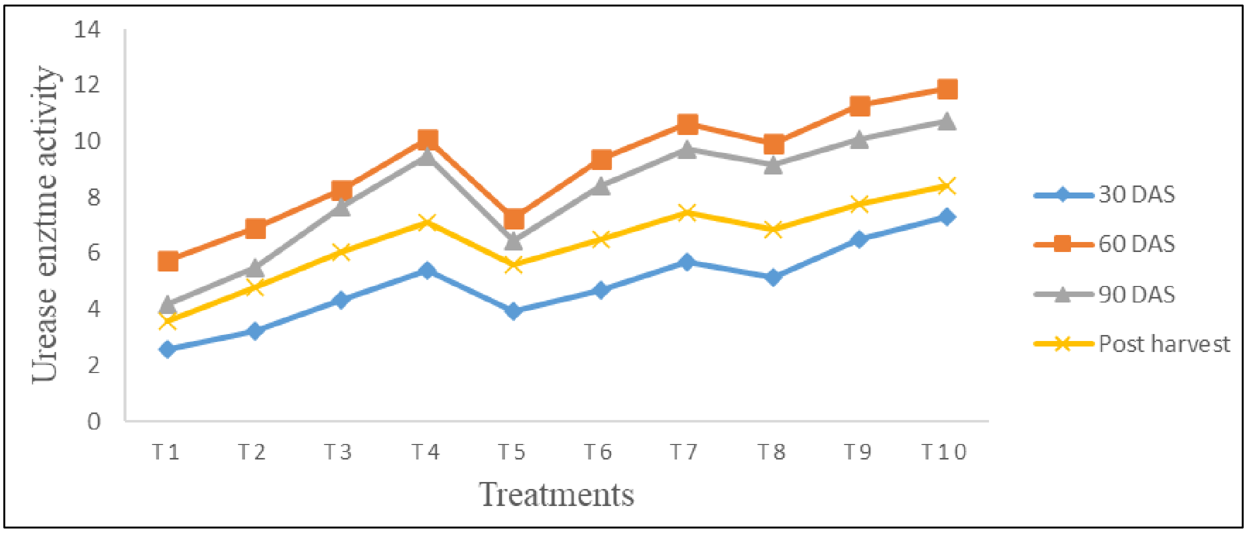

Fig 1: Combined effect of organic manures and pyroligneous acid on soil urease activity (µg NH_4^+ released g^{-1} hr^{-1}) CD test at 5%level of significance.

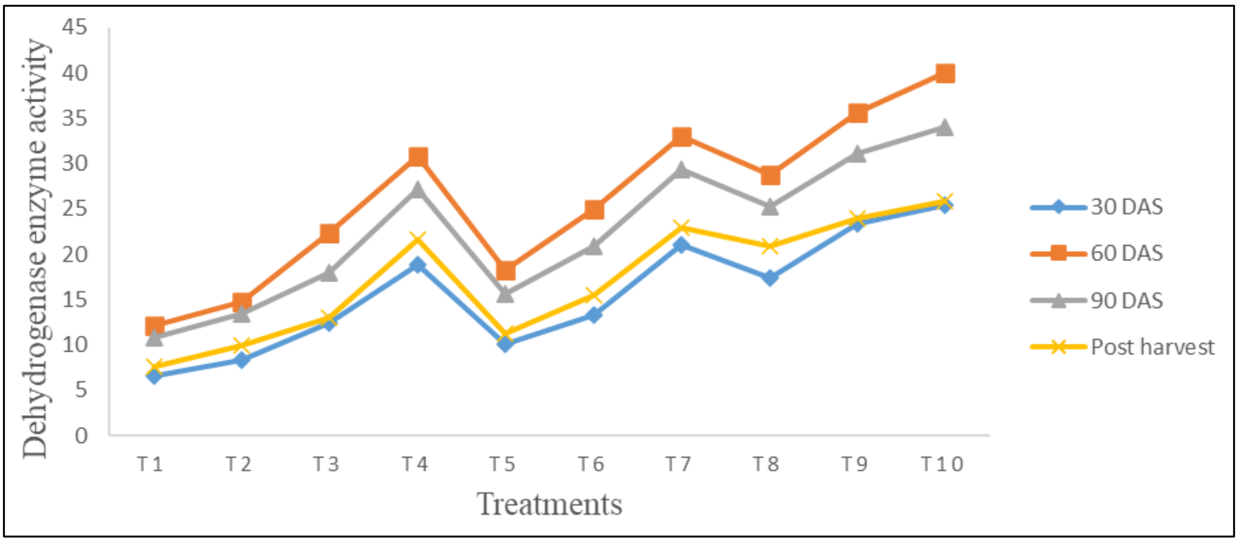

Fig 2: Combined effect of organic manures and pyroligneous acid on soil dehydrogenase activity (µg of TPF g^{-1}) CD test at 5% level of significance

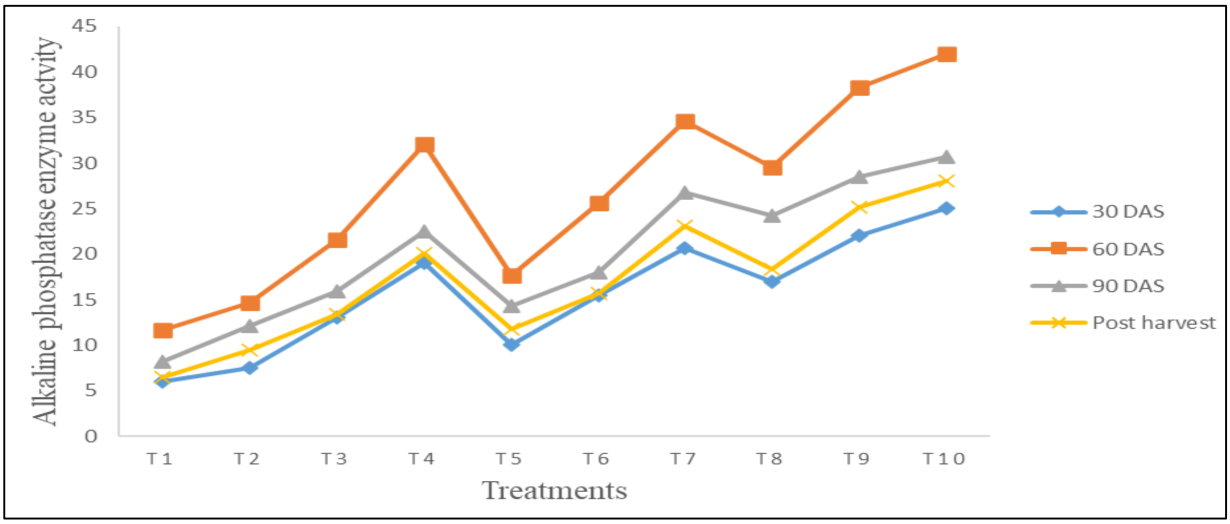

Fig 3: Combined effect of organic manures and pyroligneous acid on soil alkaline phosphatase activity (µg of PNPP g^{-1}) CD test at 5%level of significance.

Conclusion

From the present study, it can be concluded that addition of organic manure along with pyroligneous acid as soil drenching helped in increasing soil biological properties which is considered as good indicator of high soil quality. Nannipieri and Kandeler (2002) [14] reported that addition of vermiwash could have changed the soil environment by improving the rhizosphere microflora in synthesizing the enzymes or metabolites that could alter the integrity of root cells or the permeability of their membrane promoting significant increase in root exudates. Higher microbial and enzyme activity is a positive sign of soil fertility and health. In this study, enhanced enzyme activities viz., the soil dehydrogenase, phosphatase and urease were direct indicators of enhanced microbial activity in the root region which was supported by the increased microbial population that was recorded. Higher organic C of the soil due to addition of organic manures viz., FYM and vermicompost together with PA might have increased microbial population or biomass and ultimately the soil enzyme activity vital in nutrient transformation and supply in soil.

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