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Seasonal variation of Soil Biological properties in Castor (*Ricinus communis* L.) cultivated soils: A possible index towards soil fertility

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Abstract

Castor (Ricinus communis L) is a primary host plant of Eri silkworm, a Lepidopteron insect which is responsible for producing sericin. The commercial production of Eri silk is mostly confined to the Northeast India and provide livelihood to poor farmers. The soils of Castor growing areas are highly rich in microbial diversity. Measuring soil respiration, soil dehydrogenase and phosphatase activity is an important aspect to estimate soil biological properties as it acts as a biological indicator towards soil fertility. Eight soil samples were collected from two plots of castor cultivated land of farm no. 1, Central Silk Board, Lahdoigarh, Jorhat, Assam. The average pH of the soil sample was slightly acidic (pH 6.5) and soil biological property of the sample was analyzed by CO_2 evolution, soil dehydrogenase and acid phosphatase activity. The soil respiration activity was found more in the samples of plot no 8 than in plot no. 10 for different time intervals during the summer season. Whereas, the higher soil dehydrogenase and acid phosphatase activity was found in plot no. 10 than the samples of plot no.8. Moreover, samples of both the plots showed better biological activities in the summer in comparison to the other seasons with a positive correlation to environmental parameters indicating that soil biological activities vary with seasons throughout the year. The analysis was carried out as a benchmark survey for selection of experimental plots for application of biofertilizer input and subsequent formulation of an INM package for sustainable castor cultivation in ericulture.

Keywords: Castor, sericin, soil respiration, dehydrogenase, acid phosphatase.

Introduction

Soil although is non living, but it seems to behave as a matured living creature because of the different chemical reactions that take place in it. Enzymes produced by soil microorganism play a crucial role in the soil biological transformations. This is due to the fact that, enzymes are one of the members responsible for the various nutrient cycles. The amount of inorganic matters released into the soil by the enzymes has got a very interesting correlation with the fertility of various types of soil.

Castor (*Ricinus communis* L.) is a primary food plant of eri silkworm and extensively used by farmers for ericulture. The eri silkworm *Samia ricini* Donovan is a multivoltine and polyphagous sericin producing insect. Eri culture is a traditional agro-based small scale industry, primarily practiced to meet the partial need of warm clothing. Eri silkworm significantly contributes to the Indian commercial silk production which is mostly confined to the Brahmaputra valley of Assam in the tribal inhabited districts[1]. Approximately, 1.3 lakh families with plantation area of 26000 hectares are involved in ericulture in northeastern region of this country[2].

Castor growing soils have got a great variability in the biological properties based on microbial count, macrobial organisms and other organic materials in particular. Soil microorganism's experiences microbial degradation with the help of organic substrates released by the plants by production of oxido-reducing enzymes[3,4,5]. Soil plant root eco zone has been a ripe area of research since time immemorial[6]. Microbial population also helps to determine the plant productivity by analyzing different soil processes[7].

Measuring soil carbon dioxide evolution is an important aspect to estimate soil biological properties of a cultivated soil as it acts as a biological indicator towards soil fertility. Estimation of soil CO_2 evolution has been a long time process in quantifying the quality of a soil sample on the basis of microbial activities[8]. Similarly, soil dehydrogenase analysis also works as a tool in determining the fertility of soil. The various microbe origin phosphatases play a key role in solubilization of inorganic phosphates in soil. Phosphatase can be used as a major parameter for analyzing soil fertility as it is heterogeneous in nature[9].

In the present study, the soil biological properties of a castor cultivation field in different season is taken into account for input of biofertilizer consortium which is carried out by analyzing soil respiration, soil dehydrogenase and acid phosphatase activity.

Material and Methods

Sample site and collection: The experimental castor cultivation Plot no. 8 and 10 of Central Silk Board, Jorhat, Assam, India are

geographically located at latitude [$26^0$47'31"N] and longitude [$94^0$20'5"E]. The mean annual temperature range of the location is 8 - 36 ^{0}C and average annual erratic rainfall is 2029 mm. Eight samples were collected randomly from the top soil (0-30 cm depth) during each season i.e. Spring, Summer, Autumn and Winter throughout the year 2012-13. The soil samples were initially weighed by digital balance with the poly bags and brought to the laboratory in every season and the soil pH of the samples were recorded by standard method[10].

Soil respiration analysis: Soil respiration analysis was performed by release of CO_2 entrapped in NaOH solution followed by $BaCl_2$ precipitation[11]. For this, 100 g of soil samples were placed into a glass jar with three replicates. Test tube containing 10ml of 0.1N NaOH was hung inside each glass jar with the help of a cotton thread. The jar is then sealed with a rubber stopper and molten wax and allowed to incubate at 30 ^{0}C. At the end of the incubation period, the content of the tube is transferred to a 250 ml flask and immediately added 5ml of saturated $BaCl_2$ to precipitate the $BaCO_3$. The residue content of NaOH in the flask is measured by titration against 0.1N HCl using Phenolaphthalein as an indicator. The CO_2 evolution was determined by calculating the CO_2 entrapped by NaOH and utilized for precipitation of $BaCO_3$.

Soil Dehydrogenase analysis: Soil Dehydrogenase activity was analyzed as per the standard method described earlier [12]. Here, 20 g of air dried soil sample was mixed with 0.2 g of $CaCO_3$ and placed 6 g of this mixture in test tube with three replications[13]. Then, 1ml of 3% aqueous solution of 2, 3, 5-triphenyl tetrazolium chloride (TTC) and 2.5 ml of distilled water was added to the tubes[14]. The content of the tubes were mixed thoroughly with a glass rod and incubated at 37^{0}C with proper sealing for 24 hours. After incubation, 10 ml of methanol was added and gently shaked for 1 minute and filtered into 100 ml volumetric flask by using additional methanol. The intensity of the red colour was measured by spectrophotometer (Systronic 2202) at 485 nm against methanol as blank. Triphenyl formazan produced from TTC by dehydrogenase activity was estimated with reference to the calibration graph prepared from TPF standards.

Acid phosphatase activity: Acid phosphatase assay was performed by following standard protocol[15]. Placed 1 g of soil in 50 ml Erlenmeyer flask and added 0.2 ml of Toluene, 4 ml of Modified Universal Buffer (pH 6.5), 1 ml of p-nitrophenyl phosphate solution made in the same buffer and swirl the flask for a few seconds to mix its contents[16]. The flasks were sealed and placed in an incubator at 37°C for 1 hour[17]. The stopper was removed and added 1ml of 0.5M $CaCl_2$ and 4ml of 0.5N NaOH and swirl the flask for a few seconds and filter (Whatman no.2) the soil suspension. The yellow colour intensity of the filtrate due to the presence of p-nitrophenol was measured with UV-vis spectrophotometer at wavelength 420 nm. The p-nitrophenol produced by phosphatase activity was estimated by reference to a calibration graph plotted from the standard p-nitrophenol (Hi-media Ltd, Mumbai). The results recorded were the average of three independent replications with standard deviations for each sample.

Statistical analysis: All experimental data are the arithmetic mean of independent replications including the environmental parameters during 2012-13 for seasonal variation. The average data of each sample for seasonal variation of soil respiration activity, dehydrogenase and acid phosphatase activity were calculated and tested for standard deviation. Determination of correlation co-efficient at 0.01/0.05 significance level among the soil biological properties of the plots, environmental temperature and relative humidity was carried out by Statistical Analysis System (SAS) and with the help of statistical software programme 'SPSS version 16'.

Results and Discussion

The soil pH of the collected samples from two plots was recorded within the range of 6.3 to 6.7. The average soil respiration activity in both Plot no.8 and 10, per 100 g of soil was found more in the summer season as compared to that of the others at different time intervals i.e. 24h (18.28 mg/100g), 48h (18.97 mg/100g) and 72h (19.54 mg/100g), respectively (figure- 1). Similarly, the amount of carbon released calculated out from the evolved CO_2 in different time interval for both the plots was also higher (4.72 mg/100g) in summer (figure- 2) and followed by winter (4.14 mg/100g), spring (3.84 mg/100g) and autumn (3.59 mg/100g), respectively. Irrespective to the season, the results revealed that the amount of CO_2 evolution increases with prolonged incubation period. Plot wise evaluation of soil respiration showed better activity in Plot no.8 than Plot no. 10. Positive co-relation was observed during statistical analysis between the environmental parameters and soil respiration in different seasons (table- 1).

The average soil dehydrogenase activity (DH) in both the experimental plots was found more during summer season and that was followed by activity during autumn, spring and winter season, respectively (figure- 3). The comparative study showed better DH activity in Plot 10 than that of Plot 8 throughout the year. Significantly, highest DH activity in Plot no 10 (0.796 µg/10ml) and 8 (0.535 µg/10ml) was recorded during summer season, whereas the lowest activity was found in winter season both in Plot no 10 (0.553 µg/10ml) and Plot 8 (0.315 µg/10ml). The statistical analysis showed positive co-relation between the environmental parameters (temperature and humidity) and soil DH activity in various seasons for both the plots (table- 2).

Acid phosphatase (AP) activity was also estimated in spring, summer, autumn and winter seasons for the soil samples of Plot no.10 and 8 (figure- 4). Although AP activity is more during summer season, no significant ($p > 0.05$) difference was observed in all the seasons for both the plots. However, in Plot no.10 (35.06 µg/ml) the AP activity is significantly more in comparison to Plot no. 8 (32.45 µg/ml), especially in summer.

Lowest AP activity was found in Plot no. 10 (30.16 µg/ml) and Plot no. 8 (28.63 µg/ml) was found in autumn season. So far as statistical analysis is concerned, positive co-relation was observed between the environmental parameter and AP activity (table-2).

Table-1
Correlation of soil respiration and amount of released carbon with environmental parameters in various seasons of the year

Parameter	Correlation/ Significance	CO_2 evolution (mg/100g)		Carbon released (mg/100g)	
		Plot 8	Plot 10	Plot 8	Plot 10
Temperature (^{0}C)	Correlation	0.043	0.062	0.043	0.058
	Significance (Level: 0.01)	0.957	0.938	0.957	0.942
Humidity (%)	Correlation	0.241	0.274	0.245	0.267
	Significance (Level: 0.01)	0.759	0.726	0.755	0.733

Table-2
Correlation of soil dehydrogenase and acid phosphatase activity with environmental parameters in various seasons of the year

Parameter	Correlation/ Significance	Soil dehydrogenase (µg/10ml)		Acid phosphatase (µg/ml)	
		Plot 8	Plot 10	Plot 8	Plot 10
Temperature (^{0}C)	Correlation	0.835	0.904	0.258	0.136
	Significance (Level: 0.05)	0.165	0.096	0.742	0.864
Humidity (%)	Correlation	0.566	0.221	0.322	0.241
	Significance (Level: 0.05)	0.434	0.779	0.678	0.759

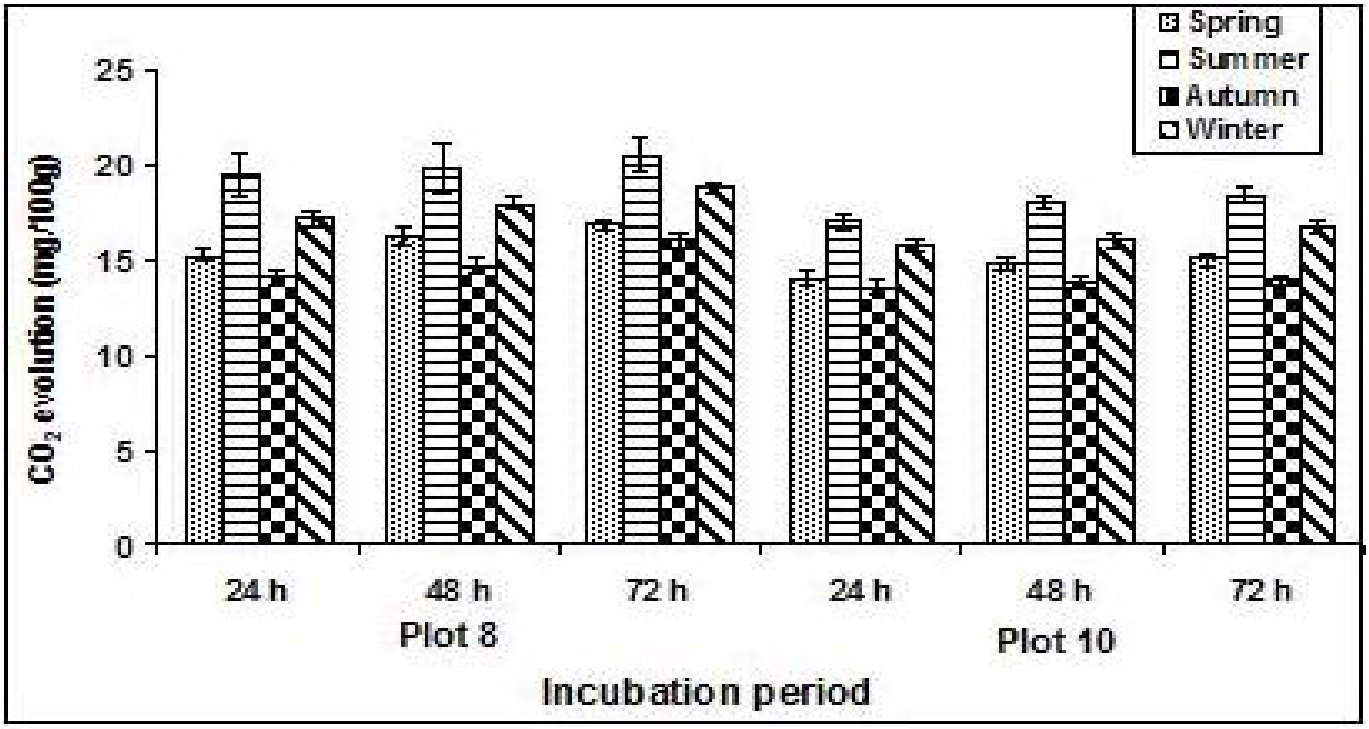

Figure- 1
CO_2 evolution in soil samples of Plot No. 8 and 10 at different time intervals in various seasons

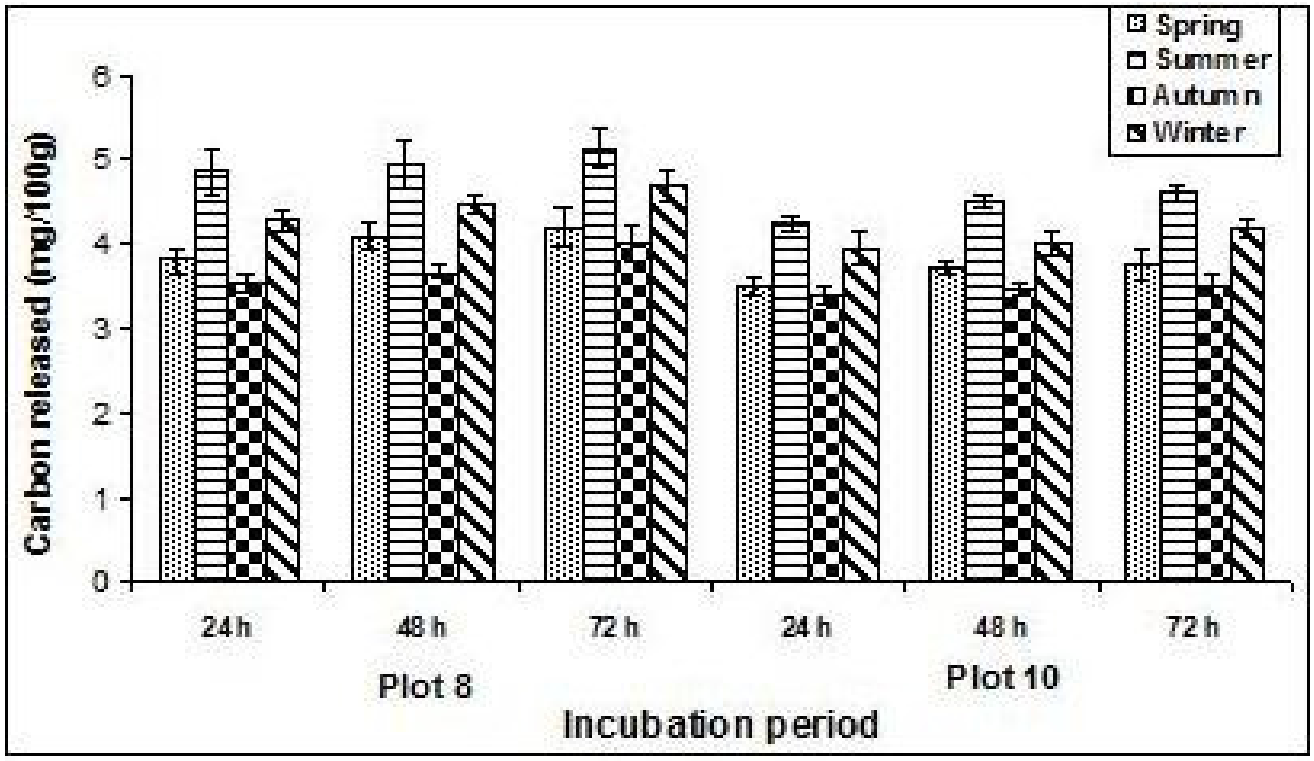

Figure-2
Amount of released carbon in soil samples of Plot No. 8 and 10 at different time intervals in various seasons

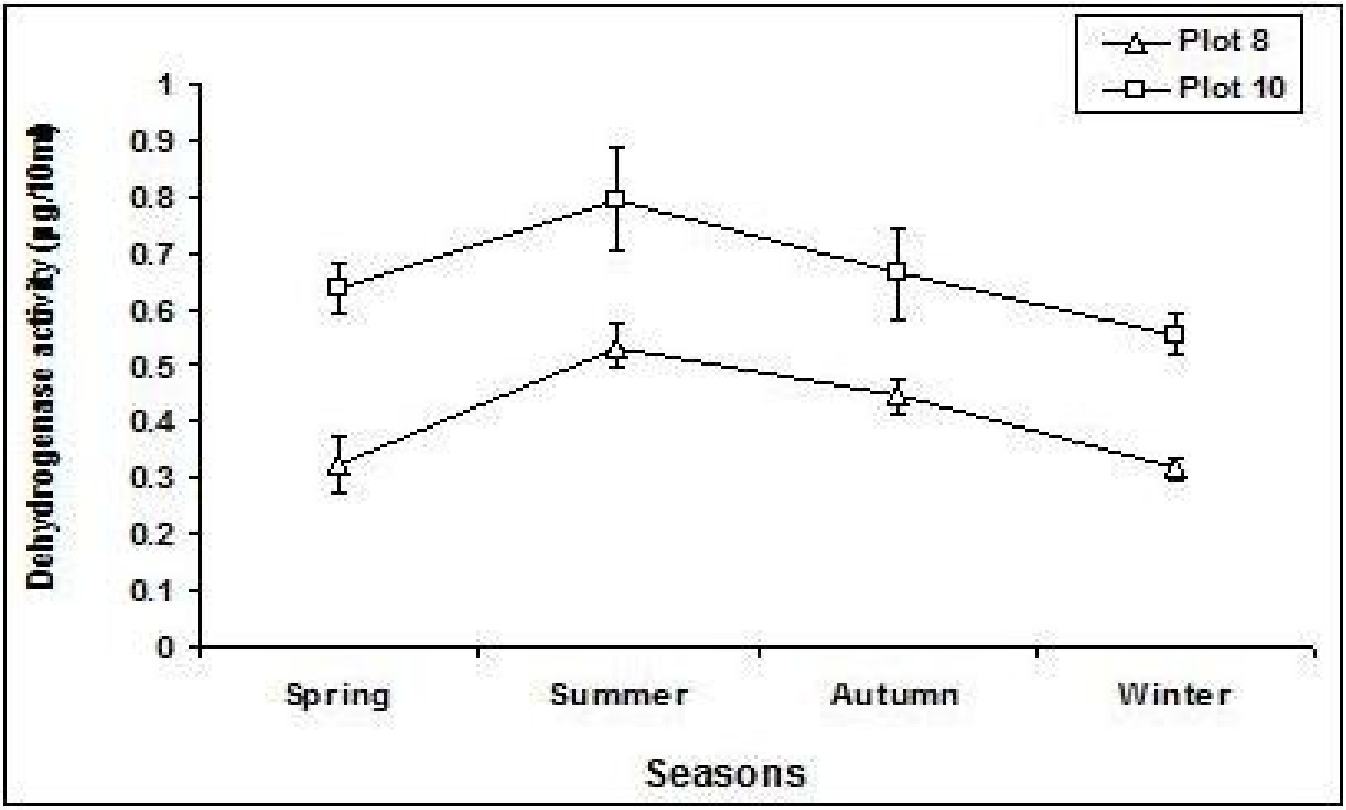

Figure-3
Dehydrogenase activity in soil samples of Plot No. 8 and 10 in various seasons

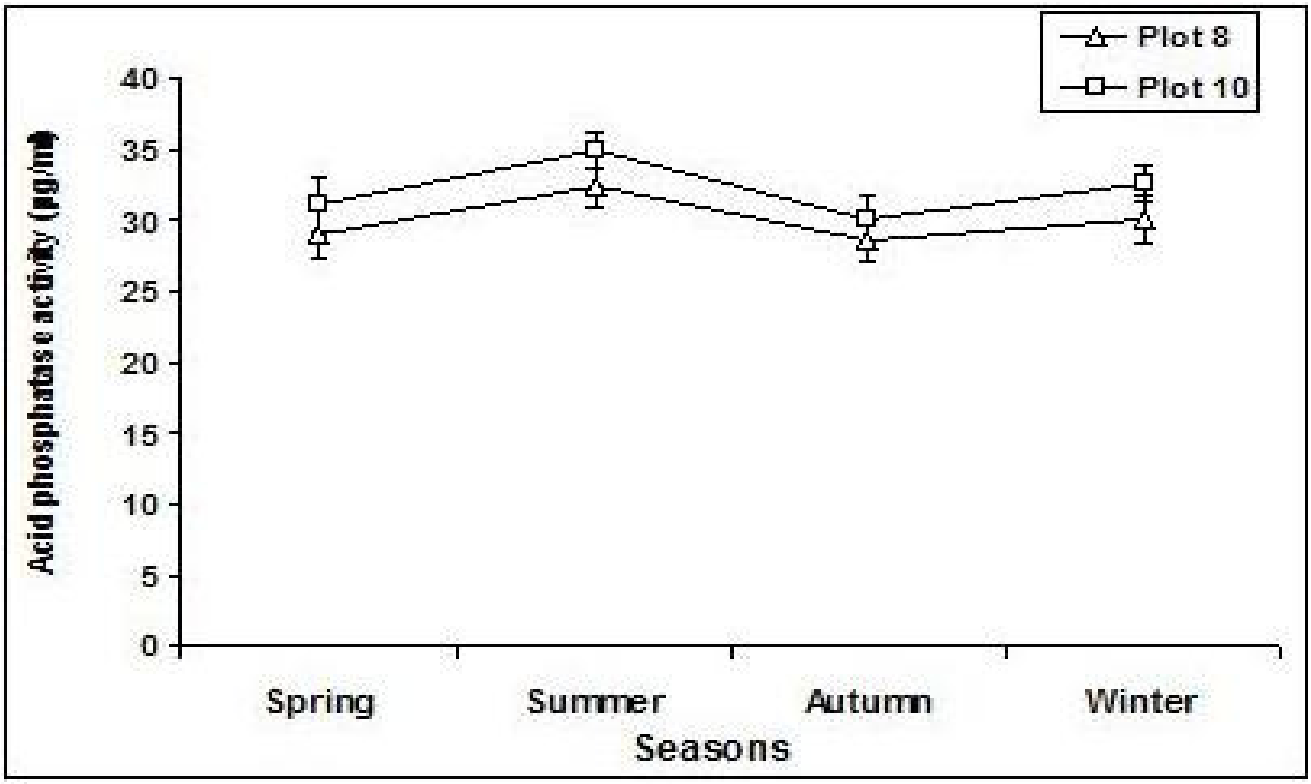

Figure-4
Acid Phosphatase activity in soil samples of Plot No. 8 and 10 in various seasons

Discussion: Soil is the store house of a variety of nutrients needed by a plant to grow and develop. Rhizosphere bears a versatile environment of acute plant microbe interactions which further help them in acquiring the essential nutrients from a nutrient pool[18]. These nutrients are made available to them through interactions between roots of plants and the microflora present in the soil. More the microbial activity more is the fertility of soil[8]. The enormity of soil microbial activities through enzymes and nutrients bioavailability determines the health and productivity standards of soil towards the sustainable agriculture. In order to gauge fertility of soil, certain tests are to be conducted. This study was conducted to determine the soil biological properties of two agricultural plots during different seasons by soil respiration, soil dehydrogenase and acid phosphatase activity in relation to environmental parameters.

Soil respiration has got a close relationship with photosynthesis and further translocation of photosynthates to the root[19]. Root respiration ranges from 33 to 60% of the total respiration of soil[20, 21] and rest of the respiration activity performed by ecto-mycorrhizal fungi and soil microorganisms [22, 23]. In our study, the seasonal variation influences the microbial soil respiration activities as well as release of C in both the experimental plots. The C content is higher in summer as compared to the other seasons. Similar report about the high C_{mic} content under wheat-millet cropping system in summer[24] was also been known.

Soil dehydrogenase is an intracellular enzyme involved in microbial respiratory metabolism and considered for nitrate reduction[25]. Dehydrogenase assay is a sensitive indicator of environmental stress and has been used for the assessment of seasonal variation of microbial activities in agriculture[3,25]. Occurrence of soil dehydrogenase activity in our soil samples proved the active colonization of Nitrogen Fixing bacteria, which produce available nitrogen for plant uptake. Enzymes in soil provide information on microbial activities which acts as a sensor to study the effects of environmental changes of soil fertility[26-29]. Dehydrogenase activity increases with the increasing soil respiration, which resembled to our findings[30]. The increase in soil water and temperature induced higher dehydrogenase activities[31].

Phosphatase plays an important role in maintaining and controlling the phosphorus cycle through soil. The average pH of the soil samples was 6.5, which is suitable for colonization of soil microflora, especially the phosphate solubilizing bacteria for mobilization of inorganic phosphate[32]. Our results showed higher phosphatase activity in summer with a positive correlation with temperature and humidity. However, phosphatase activity is less sensitive to seasonal variation[19] and no observations were made in any difference in P_{mic} contents in different season[33]. In the contrary, the increase of microbiological activities in high temperature and soil water during summer season was also reported[34]. In addition, unlike

the dehydrogenase activity, phosphate activity had no relation to soil respiration and phosphatase activity considerably correlated with the supplementary phosphorus[19].

Conclusion

The study was conducted as a benchmark survey towards the formulation of an INM package for castor plantation with special reference to the biofertilizer input. Based on the results, the soil biological property is richer in Plot no. 10 as compared to Plot no. 8. Seasonal variation of temperature and relative humidity is positively correlated with the soil microbiological activity. It may be considered that the summer season induces better microbial soil respiration, dehydrogenase and phosphatase activity for sustainable castor cultivation in ericulture.

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