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Potential of some selected soil fungi in relation to litter decomposition in tea agroecosystem

DEEPTI MALA SINGHA*, B.K. DUTTA[1] AND D. C. RAY[2]
[1]Microbial and Agricultural Ecology & Biodiversity Conservation Laboratory,
[2] Insect Ecology and Soil Biology Laboratory.
Department of Ecology and Environmental Science, Assam University, Silchar 78801, Assam

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Decomposition of leaf litter is a major source of nutrients in tea agroecosystem. The leaf litter is broken down by the insects and microbial decomposers. Decomposition refers to the processes that convert dead organic matter into smaller and simpler compounds leading to mineralization. The rate of decomposition is influenced by many factors. In the present work, leaf litter decomposition was measured using the Nylon bag technique. Six fungal species were isolated from tea soil and selected for litter decomposition study, viz, *Aspergillus niger, Aspergillus flavus, Trichoderma viride, Trichoderma harzianum, Penicillium rubrum* and *Penicillium frequentans*. They were cultured *in vitro* and introduced in the tea leaf litter to know their potential in the process of litter decomposition. Litter decomposition rate was found to be higher in the *Aspergillus flavus* amended litter followed by *Penicillium rubrum and Trichoderma harzianum* respectively as compared to other selected species of fungi and control. Potential of tea soil mycoflora in relation to litter decomposition in tea agroecosystem has been discussed.

Key words: Litter decomposition, tea agroecosystem, soil mycoflora

INTRODUCTION

Plant litter decomposition is an important biological process driven by a range of complex and interacting physical factors, such as climate, substrate, soil organisms, and physical and chemical properties of soil (Dyer *et al.*, 1990, Berg *et al.*, 1993, Singh *et al.*, 1999, Pausas *et al.*, 2004). Vitousek *et al.* (1994) have suggested that decomposition rate decreases exponentially as temperature falls along with the elevation gradients, but there are many other factors that can alter to control litter decomposition in all the terrestrial ecosystems, including climate, edaphic factors, resource quality, fauna, and microbes. As decomposition is a biological process carried out primarily by bacteria and fungi, its rate is expected to be affected by temperature and soil moisture.

Decomposition is the progressive dismantling of organic materials into its simplest constituents. In nature, decomposition is mediated mainly by soil microorganisms, which derive energy and nutrients from the process. It usually occurs not as a single step, but as a cascade. The substrates for decomposition include a wide range of materials, forming a continuum from recently added plant litter to very stable, humified organic matter. Decomposition can be sub-divided into two processes: primary decomposition, involving the breakdown of fresh litter and secondary decomposition, involving the progressive breakdown of decomposition product. Fresh material, usually plant litter, is converted to altered form with the release of CO_2, NH_4 and

*email : candela_rj@yahoo.co.in

other inorganic compounds (Couteaux *et al.*, 1995). The three major factors which influence the decomposition process are climate, litter quality, soil and litter biota (Swift *et al*, 1979; Coûteaux *et al*, 1995). Climate and litter quality are considered to be the most important regulators of decomposition on a global scale, but more locally soil fauna becomes important, especially where temperature and moisture are not limiting factors. Biotic factors, especially the presence of arthropods and fungi, are much more important in the humid tropics (Lavelle *et al*, 1993;). Fungi are likely to be more important than bacteria in surface litter decomposition because they are physiologically better adapted to invading coarse litter with hyphae and withstanding dessication, whereas bacteria potentially perform better with finer litter fragments with greater surface area and increased water retention (Beare *et al*, 1992). Many factors like temperature, rainfall, pH of the soil, altitude etc affect the decomposition rate, the diverse chemical composition of different litters plays a crucial role in determining the rate of decomposition and nutrient cycling (Dutta *et al.*, 2001). The litter or detritus resource is broken down by the combined action of the decomposer community consisting predominantly of microorganisms i.e., fungi and bacteria.

Keeping the above in view, some observations have been made on the litter decomposition potential of some fungi isolated from the soil under the agroclimatic condition of Barak Valley of Assam.

MATERIALS AND METHODS

Study site

The present work was conducted from May 2012 to October 2012. The experimental site was in Rosekandy tea estate of Barak Valley, Assam, India, which is surrounded by N.C. Hills and Jaintia Hills in the North, in the East of Manipur and in the West of Tripura and South of Bangladesh. The area has an altitude of 26-30m above M.S.L. and falls under $24^0 8'$ N latitude and 92^0 15' E longitude. It is observed that the average rainfall received by the area is 95 mm during the month of December-February. The temperature regimes of the area accompanied by a fairly high relative humidity reveal that the area experiences humid subtropical climate.

Isolation of the soil microorganisms and their identification

Technique of isolation of microorganisms was done by Soil Dilution Plate Method based upon the technique used by Waksman (1922) and Timonin (1940). Counts of suitable dilutions made in three replicates and average count of the microbe was recorded. Dilution of 1:10,000 was made for fungi. The soil microorganisms were grown aseptically in the laboratory condition in suitable agar media maintaining favourable temperature (25 ± 2ºC) and incubation period (6-7days). Some species of fungi were isolated and maintained in the culture tube containing suitable agar media and they were identified with the help of standard literature (Gilman, 1956; Barnett and Hunter, 1972; Subramanium, 1971).

Six predominant isolates of fungi selected for the experiment were *Aspergillus niger, Aspergillus flavus, Trichoderma viride, Trichoderma harzianum, Penicillium rubrum and P. frequentans.*

Subsequently, the isolated fungal cultures were inoculated in Czapek Dox liquid medium (500 ml) for experimentation and kept in an incubator (25 ± 2ºC) for a week to allow them to grow on the surface of the liquid culture medium.

Study on the rate of litter decomposition

For the study of decomposition rate of litter, Nylon bag technique (Gilbert and Bocock, 1960) was adopted. The mesh size taken was 2 mm, small enough to cause major loss of litter samples, yet large enough to permit aerobic microbial activity and free entry of soil micro and mesofauna. For this experiment, air dried primary litter from tea plants i.e. *Camellia sinensis* (L) O. Kuntz were used. Six microorganisms selected for this experiment were i.e. *A. niger, A. flavus, T. viride, T.harzianum, P. rubrum* and *P. frequentans*. For the experiment, 126 nylon bags (mesh) were made. Five g of the air dried litter from tea plants i.e. *Camellia sinensis* (L) O. Kuntz were weighed and filled in the bags which were then stitched properly.

These 126 bags were divided into six groups of 21 bags each. The litter bags were poured and mixed with broth culture of the six selected fungal species separately, i.e., *A. niger*, *A. flavus*, *T. viride*, *T. harzianum*, *P. rubrum* and *P. frequentans*. In control, only tap water was applied.

The above mentioned bags were randomly dispersed in the Tea agro ecosystem on the tea field

soil. These bags were kept in such a way that each group has three replicates of each organism treated bags and the control. After a month, first 21 bags were collected from the field, washed carefully with water, oven dried for 4-5 days at 60ºC and then the litter was weighed and calculated. In the same manner subsequently other bags were also collected after completion of every month for six months, the same process was repeated, the results were noted down and the rate of litter decomposition was determined.

Weight loss over time

Monthly weight loss (g/month) of decomposing litter was determined from the difference between the weights remaining in the litterbags in each month.

Negative exponential decay model (Olson, 1963) was used to calculate the weight loss over time as follows: $L/L_0 = \exp(-kt)$ where, L = is the weight remaining at time t, L_0 = the initial weight, exp = the base of natual logarithm, k = the decay rate coefficient and t = the time (year)

The required time for 50% (t_{50}) and 99% (t_{99}) decay was calculated as $t_{50} = 0.693/k$ and $t_{99} = 5/k$.

RESULTS AND DISCUSSION

Decomposition is the process that accounts for a huge majority of the biological carbon processing on earth. It is carried out primarily by bacteria and fungi. The term decomposition is defined as the biological disintegration of dead organic matter into simple inorganic forms by the process of mineralization of complex organic compounds (Sharma *et al*, 1995). The decomposition rate is strongly influenced by climatic conditions, an initial chemical composition of the litter. The important parameters observed include soil pH and moisture content as shown in the Table 1.

From the above table it can be seen that the pH value of tea soil ranges between 5.29 to 6.06 from May to October and the highest moisture content is seen in the month of August and the lowest in the month of October.

Litter decomposition was studied through litter bag technique after placing specific fungal broth culture. The objective was to observe whether along with other environmental factors, any special fungal species can hasten the process of litter decomposition. In the above result, it can be seen that the rate of litter decomposition in the control was comparatively slower compared to other mycoorganism treatments, which showed that the inoculation of soil fungi (decomposers) to the litter enhanced the decomposition process. The data generated during the investigation revealed that the various microbes can cause varied degree of litter decomposition, when applied on them separately.

Table 1 : Physiological parameters

Month	Soil pH	Soil moisture content (%)
May	6.01	24.01
June	6.04	22.05
July	6.06	21.38
August	5.88	27.35
September	5.58	21.72
October	5.29	17.35

The rate of litter decomposition was found to be highest in *A. flavus* treated litter followed by *P. rubrum* and *T. harzianum*. (Table 2a). They were found to be good decomposers, while *A.niger, P.frequentans* and *T.viride* were having moderate capability of decomposing. Lavelle *et al.* (1993) had opined that litter decomposition in the warm and humid condition is more rapid as the prevailing environmental conditions are conducive for rapid decay. Swift *et al.* (1979) and Anderson and Ingram (1993) reported higher initial weight loss with the litter bag technique. The maximum weight loss may have been contributed by the high rainfall, relative humidity and temperature. Most of the authors have reported that, higher loss of litter mass occurred during the rainy season as compared to the dry season (Facelli and Pickett, 1991; Vucetich *et al.*, 2000). The maximum weight loss recorded may be related to the favourable conditions for rate of litter decaying and soil moisture contents, high relative humidity and temperature, all indirectly favouring the soil biological activity. The results of the higher decay rates during the wet months conform with the result of Pandey and Singh, (1980); Isaac and Nair (2005) and are attributed to rapid microbial activity and accentuated leaching due to

Table 2a : Amount of weight loss by the fungal isolate when inoculated with tea leaf litter (month)

Month	Organisms inoculated						
	Control	*Aspergillus niger*	*Aspergillus flavus*	*Penicillium rubrum*	*Penicillium frequentans*	*Trichoderma viride*	*Trichoderma harzianum*
May	3.67±0.06	3.52±0.13	3.12±0.16	3.25±0.22	3±0.15	3.2±0.18	3.36±0.24
June	2.84±0.16	2.75±0.14	2.8±0.12	2.55±0.28	2.44±0.22	2.75±0.17	2.9±0.16
July	2.35±0.32	1.99±0.14	2.21±0.18	2.06±0.31	2.02±0.10	2.23±0.41	2.37±0.36
August	2.2±0.25	1.61±1.03	0.96±0.05	0.73±0.10	1.56±0.29	0.77±0.03	0.86±0.08
September	1.35±0.37	0.87±0.09	0.44±0.22	0.37±0.20	0.73±0.24	0.74±0.22	0.57±0.06
October	1.17±0.18	0.86±0.18	0.25±0.14	0.3±0.13	0.37±0.21	0.58±0.18	0.56±0.14
F value	13.776*	5.716**	61.912*	30.950*	19.559*	25.028*	38.387*

Here, * indicates that the P value is significant at 0.001 level.
** indicates that the P value is significant at 0.05 level.

Table 2b : Determination of k value (Decay rate coeffecient), t_{50} and t_{99} (as per Olson, 1963)

Treatments	k value (Decay rate coefficient)	t_{50} (50% decay)	t_{99} (99% decay)
Control	2.94	85.88	619.64
Aspergillus niger	3.56	70.86	511.28
Aspergillus flavus	6.07	41.63	300.42
Trichoderma viride	4.36	57.9	417.79
Trichoderma harzianum	4.43	56.97	411.09
Penicillium rubrum	5.7	44.33	319.89
Penicillium frequentans	4.26	59.29	427.81

rainfall. Organic litter follows an exponential decay curve. The formula for this decay is L/Lo = exp (-kt).

Here, k , in equations determining the amount of litter remaining after a certain period of time. The higher k value was found in *A.flavus* (k = 6.07) followed by *P.rubrum* (k= 5.7), *T.harzianum* (k= 4.43), *T.viride* (k= 4.36), *P.frequentans* (k= 4.26), *A.niger* (k = 3.56) and Control (k = 2.94). From the above statement it was observed that, k- value of *A.flavus* is higher which shows that *A.flavus* can hasten the process of litter decomposition faster compared to other mycoorganism treatments.Table 2a Isaac and Nair (2005) documented the litter of *Mangifera indica* (L) and *Artocarpus heterophyllus* (Lamk.) to have k- value of 2.35 and 3.05 from the homegarden of warm humid climate of Kerala whereas, Jamaludheen and Kumar (1999) recorded the k-value of later one was 0.22 from monoculture woodlots of Kerala. Here the fungal species i.e., *A. flavus* amended tea leaf litter have the k-value 6.07, which is higher than the single species decomposition studied by Jamaludheen and Kumar (1999); Isaac and Nair (2005).

The required time for 50% (t_{50}) decay (in days) was found to be t_{50}= 85.88, t_{50}= 70.86, t_{50}= 41.63, t_{50}= 57.9, t_{50}= 56.97, t_{50}= 44.33, t_{50}= 59.29 and time required for 99% (t_{99}) decay was found to be t_{99}= 619.64, t_{99}= 511.28, t_{99}= 300.42, t_{99}= 417.79, t_{99}= 411.09, t_{99}= 319.89 and t_{99}= 427.81 for control, *A.niger, A.flavus, T. viride, T.harzianum, P.rubrum* and *P.frequentans* respectively (Table 2a) (Calculated as per Olson,1963). From the above result, it is seen that the t_{50} and t^{99} value of *A. flavus* shows less number of days for decomposition and hence, can hasten the process of litter decomposition compared to other fungal species used in the experiment. The predicted half-life (time taken to decompose 50% of the initial mass) and 99% decay period also shows variation from one fungal species to another and is an indication of the persistence of the litter on the soil surface. Prevalence of climatic conditions and different initial litter chemistry can be attributed for such differences. Lower

Table 3 : Rate of tea leaf litter decomposition (%)

Month	Control (%)	*Aspergillus niger* (%)	*Aspergillus flavus*(%)	*Trichoderma viride* (%)	*Trichoderma harzianum* (%)	*Penicillium rubrum* (%)	*Penicillium frequentans* (%)
May	26.6	29.6	37.6	36	32.8	35	40
June	43.2	45	44	45	42	49	51.2
July	53	60.2	55.8	55.4	52.6	58.8	59.6
August	56	67.8	80.8	84.6	82.8	85.4	68.8
September	73	82.6	91.2	85.2	88.6	92.6	85.4
October	76.6	82.8	95	88.4	88.8	94	87.8

the value, faster will be the decay and nutrient release (Issac and Nair, 2005).

CONCLUSION

Among the fungi inoculated with the leaf litter, *A. flavus* showed the highest percentage of decomposition as reflected by the weight loss in the said treated litter followed by *P. rubrum* and *T. harzianum* (Table 3). Although at the very onset, most of the fungi under experiment showed almost similar percentage of weight loss of the treated litter samples. But during the later part of the experiment, the above mentioned good decomposers went ahead compared to others, seems to be due to their better decomposing capability.

Faster decomposition rate by *Aspergillus* sp. and *Penicillium* sp. can be attributed to their capability of utilizing both labile and recalcitrants (Khar, 1983). The percentage weight loss caused by them was found to be faster and higher for the entire course of experiment. In the first few months, may be the absence of lignified substances resulted in the continuous higher rate of percentage weight loss (Sharma *et al.,* 1995). The decomposition constant k is primarily controlled by climate and litter quality. Higher k- values correspond to faster rates of decomposition, while lower values correspond to slow process of decay.

From the results obtained in the present work, it can be suggested that some isolates of soil fungi are good decomposers. However, the isolated soil fungi have varied potential for litter decomposition. Therefore, more genera/species of fungi may be isolated from soil and litter to find out fungi with better potential litter decomposers, with higher potential for decomposition which should contribute to faster rate of litter decomposition and nutrient recycling in the tea agroecosystem of Barak Valley at large.

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