Terrestrial and Aquatic Environmental Toxicology ©2012 Global Science Books

Influence of Tannin in Soil Adjacent to *Acacia nilotica* (L.) Willd. ex Del. on Microbial Population

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ABSTRACT

The tannin polyphenol in soil influence microbial growth. Soil fertility is mainly due to the microbial load existing in a place which is decided by the physical and chemical nature of the soil. Hence in the present study, we attempted to estimate the different microbial populations in a tannin-rich soil adjacent to *Acacia nilotica* (L.) Willd. ex Del. trees and compared the results with microbial populations present in an area where there was no tannin. The present study revealed shifts from normal counts of predominant soil microbial groups. In non-tannin soil, bacterial counts (720 CFU × 10^4/g) outnumbered fungal and actinomycete counts. Compared to other groups, fungi were the least abundant in non-tannin soils (5.8 CFU × 10^4/g). The sequence of dominance in tannin soil followed the order: fungi < actinomycetes < bacteria. A comparatively higher total microbial count was responsible for high aggregate stability and reduced bulk stability of non-tannin soil. Nitrifiers in tannin soil are inhibited by tannins to account for lower soil nitrate content (4 µg g^{-1} dry soil) compared to non tannin soil (33 µg g^{-1} dry soil). The ecological consequences of elevated tannin levels of 5% may include allelopathic responses, changes in soil quality and reduced ecosystem productivity.

Keywords: *Acacia nilotica,* allelopathy, microbes, nitrifiers, soil fertility, tannins

INTRODUCTION

Allelopathy refers to an ecological phenomenon of plant–plant interference through release of organic chemicals (allelochemicals) in the environment (Mallik 2008). Khan *et al.* (2009) claimed that many plants release allelochemicals that are dangerous for the crops and environment. A wide variety of allelochemicals are involved in interactions between plants and microbes, such as fungistasis, spore germination, antibiosis between microorganisms, development of disease symptoms, promotion of infection and host resistance to pathogens (Mallik 2008). Phenolic compounds are very harmful to organisms even at low concentrations due to its toxicity and carcinogenicity properties (Din *et al.* 2009). Phenol is a listed priority pollutant by the U.S. Environmental Protection Agency (US-EPA 2002) and is considered to be a toxic compound. Phenol may persist in air, sea water or surface water, soil or sewage (Eman *et al.* 2011). Phenolics may enter the soil through industrial effluents, tree litter or through plant leachates and root exudates. Tannins are plant-derived polyphenolic compounds that precipitate proteins, bind to metals and complex with other compounds (Halvorson and González 2008). Tannins affect the types and distribution of microorganisms present in a soil (Kraus *et al*. 2003). A number of studies demonstrated that tannins inhibit microbial activity (Baldwin *et al.* 1983; Schulz *et al*. 1992; Schimel *et al*. 1996, 1998; Fierer *et al*. 2001). Tannins could intervene by binding to either the enzyme (e.g. trypsin) or the substrate (e.g. leaf protein) or to both (Mole and Waterman 1987). Tannins in soil can affect nutrient cycling by hindering decomposition rates, complexing proteins, inducing toxicity to microbial degraders and inhibiting enzyme activities (Kraus *et al.* 2003). Tannins are known to affect the microbial biodegradation process in soil, which is the main source for the release of nutrients for plant growth and yield. Microbial activity is a potential indicator of soil quality and has the ability to predict changes in soil properties (Ruark and Zarnoch 1992; Barrett and Burke 2000). The fate of tannins in the soil and their exact action upon soil microbes is not clear. The structural type of tannin is important when its reactivity is considered (Kraus *et al*. 2003a; Nierop *et al*. 2006). The complexity and diversity of tannin structures, as well as the presence of mixtures of phenolic compounds in plants, complicate the study of tannins (Smith *et al.* 2003). Soil is the natural habitat for a myriad of microorganisms and other living forms representing numerous genera and species (Bugmann 1996). The numbers, kinds, and activities of these organisms vary from soil to soil as influenced by soil organic matter content, soil texture, pH, moisture, temperature, aeration, and other factors (Subler and Kirsch 1998; Peuke 2000; Smith *et al*. 2000). Bacteria are the most abundant microorganisms in soil, attaining 10^8-10^9/g of soil. Actinomycetes are the second most numerous microorganisms in soil, numbering 10^7-10^8/g soil, followed by fungi and algae in the range of 10^5-10^6/g and 10^4-10^5/g, respectively (Ashton and Macintosh 2002; Delgado and Follett 2002).

Acacia trees are a good source of organic matter to the soil. One of the main tenets of agroforestry is that trees maintain soil fertility (Palm 1995). Tree litter constitutes a wide range of C compounds that decompose at different rates (McTiernan *et al*. 2003; Janzen 2005). The rate of litter decomposition is determined by the quality of litter in terms of the abundance of its different components and its physical structure, as well as by environmental conditions (Fioretto *et al*. 2005; Mukhortova 2005). Though the nitrogen-fixing ability of *Acacia* tree makes it one of the most preferred species for agricultural fields (Puri *et al*. 1994), the tree also contain a high tannin content and the ecological consequences of elevated tannin levels may include microbicidal activity, changes in soil quality, allelopathic

Received: 10 February, 2011. Accepted: 22 March, 2012.

Original Research Paper

responses and reduced ecosystem productivity. Allelopathy was implicated by Al-Wakeel *et al.* (2007) and Lorenzo *et al.* (2008) when they observed that the water extracts of different *Acacia* species inhibited the germination, root and shoot length, and dry weight of different crops and weeds. Hussain *et al.* (2011) in their study carried out with the extracts of *A. melanoxylon* found that they had significantly reduced the germination and seedling growth of the native species. There is strong evidence that tree tannins play an important role in interspecies competition. In many studies the results have suggested that individual plants, due to the tannins they contain, may be important in nutrient cycling on the ecosystem level (Kraus *et al*. 2004; Nierop *et al*. 2006a). El-Khawas and Shehata (2005) observed and reported the allelopathic effects of leaf leachates of *A. nilotica* that reduced growth parameters in *Zea mays* L. (maize) and *Phaseolus vulgaris* L. (kidney bean). Al-Wakeel *et al.* (2007) reported that the higher doses of *A. nilotica* leaf residue (0.75, 1.0, 1.5, and 2%, w/w) were inhibitory to seedling growth and the effect was concentration-dependent. In Africa and the Indian subcontinent, *A. nilotica* is extensively used as a browse, timber and fire-wood species. The plant was actively spread as a shade tree along bore drains in many countries. But, because of their uncontrolled vast growth, countries like Australia have announced the plant to be a noxious weed following concern about its rapid spread. The present study investigated soil quality in terms of physico-chemical and microbiological characteristics, existing in a tannin-rich environment (5%), under *A. nilotica* trees and compared it with soil existing in a tannin-free environment, covered with grass vegetation and where occasionally maize were grown. Since microbial populations decide the fertility of soil, such studies are important to elucidate the soil quality near agroforest tree plantings with *A. nilotica* growths.

MATERIALS AND METHODS

Collection of soil samples

Two categories of soil samples *viz.*, tannin-soil (soil from around and near the region of *A. nilotica* (L.) Willd. ex Del. tree) and non-tannin soil (soil from a field where no *A. nilotica* grew) were collected from top zone within the 'A' horizon, a layer of mineral soil with most organic matter accumulation and soil lives such as earthworms, potworms (enchytraeids), arthropods, nematodes, fungi, and many species of bacteria and archaea, etc, often in close association with plant roots. Hence 'A' horizon is referred to as the biomantle (Johnson *et al*. 2005; Wilkinson and Humphreys 2005).

1. Sampling of non-tannin soil

A sampling field of 280 m^2 in pasture land with an even topography was chosen for the collection of soil samples. Systematic random sampling was done (Crepin and Johnson 1993). Five representative plots (replications) of 1 m^2 each were chosen systematically in the sampling field with a minimum of 9-m intervals between them. Five intact soil cores (≈150 cm^3) from each representative plot were randomly removed at 0-25 cm using a 5-cm diameter La Motte soil sampling corer with a removable inner tube. Sub-samples were mixed together thoroughly to produce one composite sample for each representative plot (5 plots = 5 replicates). Clean soil sample bags were used for collection of samples.

2. Sampling of tannin soil

The soil samples from under five randomly selected 40-years-old *A. nilotica* trees with a canopy diameter of 20 m were removed from the study stand. Samples were collected from five equally spaced spots approximately 4 m from around each *A. nilotica* tree trunk. The five sub-samples with similar core size as described in the previous paragraph were bulked for each tree to give a composite sample that represented that tree site (one replication) and collected in clean sample bags (Ushio *et al.* 2010). At the time of sampling, the soil temperature was 20-25°C and soil moisture content was 50-60% as determined on a gravimetric basis. The soil samples were stored in air-tight plastic containers in freezer bags at 4°C until analyzed.

Processing of soil samples

A portion of the soil sample from each study site was air dried for analyses of different physico-chemical parameters. Soil samples were spread out on a sheet of paper. Soil lumps were broken with a spatula and the soil was left to dry at room temperature for 24 h. Any roots, stones, pebbles or other foreign material was removed. When the samples were sufficiently dried to sift easily through the fingers, it was ground to pass through a 2-mm sieve (ISO 10381-6 1993). A portion of the sieved soil samples was ground in strong mortars to a fineness of 1 mm. Ground soil sample (100 g) was further ground in a strong mortar to pass through a 0.5 mm sieve for the analysis of tannins (Makkar 2003).

Physico-chemical assays

The tannin-free and tannin-rich soils were characterized with reference to physical, chemical and microbiological components.

1. Soil color and texture

In determining the soil color, generally air-dried soil samples were preferred since the presence of moisture would vary the intensity of colour of soil. A Munsell® Color Geological Rock-Color Chart 2009 Revised Washable Edition by Munsell (http://www.forestry-suppliers.com) was used for comparing soil colors. The particle size distribution in the soil profiles was done using the hydrometric method using procedures of Gee and Or (1992).

2. Dry matter (DM) determination

The partial dry matter and total dry matter of the samples were determined following the procedure of Association of Official Analytical Chemists (AOAC 1984). The Dry matter (DM) was determined by drying the samples at 105°C for 24 h.

3. pH

The pH of the soil was measured using pH meter by immersing the glass electrode in a fresh soil-water slurry (1:2.5, w/v), after equilibrating for 1 h (Peech 1965). Determination of pH and mineral N (NO_3-N and NH_4^+-N) were done immediately after the collection of soil samples.

4. Organic matter determination

The organic matter (OM) was determined as a loss in weight of the sample after incinerating (ashing) at 600°C for 3 h (AOAC 1984).

5. Carbon and Nitrogen

The organic carbon (OC) content was estimated as 58% of the organic matter (Jackson 1958). Total nitrogen (TN) estimation was done on the soil samples using the micro-Kjeldahl method (AOAC 1984).

6. Aggregate stability and bulk density

Aggregate stability was estimated by the wet sieving method (Kemper and Rosenau 1986). Bulk density of the soil samples were determined by the clod method as described by Blake and Hartge (1986) and gravimetric water content was determined as described by Gardner (1986). Gravimetric water content (q_g) is readily measured by weighing a sample of moist soil, oven drying it at 105°C for 24 h (or until the mass stops decreasing) and then reweighing the oven dry soil. Gravimetric water content is the mass of water per mass of dry soil and is then measured as: q_g = (mass moist soil – mass oven dry soil) / mass oven dry soil (Instruction manual for HydroSense Soil Water Measurement System (Revision: 7/10), © 1999-2010, Campbell Scientific, Inc.)

7. Total available water holding capacity

Water held in a soil (water content) was quantified on a gravimetric basis (g water/g soil).

8. Electrical conductivity

The saturated soil paste extract was collected in a 250-ml vacuum flask. Electrical conductivity (EC) of saturated paste extract was determined using an AC conductivity bridge which can be operated either at line frequency or 1 KHz and conductivity cell with two electrodes at a set distance of 1 cm from each other. EC is referenced to a standard temperature (25°C). Soluble salts is reported as the conductivity in the solution in mmhos/cm (=dS/m) (Soil and Plant Analysis Council 2000).

The bridge contains two fixed legs, a voltage divider range switch and a precision potentiometer which is mechanically connected to a readout dial. The bridge output is amplified and applied to the grid of an indicator tube. As the bridge output approaches zero, a rectangular shadow appears on the screen of the indicator tube. In operation, the sensitivity control, range switch and drive control are adjusted until a maximum shadow appears on the indicator tube. Conductance is thus read from the dial following instruction manual for YSI Model 31 Conductivity Bridge.

9. Tannin assay in soil

Total phenolics and tannins were measured in soil samples using Folin-Ciocalteu method (Makkar *et al.* 1993).

9.1 Extraction of tannins using 70% aqueous acetone for total tannin

Two hundred mg of dried, finely ground (0.5 mm) sample was taken in a 25 ml capacity glass beaker. Ten ml of aqueous acetone (70%) was added and the beaker was suspended in an ultrasonic water bath (Branson 3210) and subjected to ultrasonic treatment for 20 min (2 × 10 min with 5 min break in between) at room temperature. The contents of the beaker were then transferred to centrifuge tubes and subjected to centrifugation for 10 min at approximately 3000 × *g* at 4°C. The supernatant was collected and stored in ice (Makkar 2000).

9.2 Analysis of total phenols

Taken suitable aliquots of the tannin-containing extract (0.02, 0.05 and 0.1 ml) in test tubes, made up the volume to 0.5 ml with distilled water, and added 0.25 ml of the Folin-Ciocalteu reagent and then 1.25 ml of the sodium carbonate solution. Vortexed the tubes and recorded absorbance at 725 nm after 40 min. Calculated the amount of total phenols as tannic acid equivalent from the calibration curve. The total phenolic content was expressed on a dry matter basis (x%).

9.3 Removal of tannin from the tannin-containing extract

Polyvinyl polypyrrolidone (PVPP) binds tannins, thus 100 mg High molecular weight range PVPP, commercially available from Sigma (Product no. P- 6755) was weighed in a 100 × 12 mm test tube. To this, 1.0 ml distilled water and then 1.0 ml of the tannin-containing extract was added. The tube was vortexed then kept at 4°C for 15 min, vortexed again, then centrifuged at 3000 × *g* for 10 min. The supernatant was collected (Makkar *et al*. 1993). This supernatant has only simple phenolics other than tannins (the tannins would have been precipitated along with the PVPP). The phenolic content of the supernatant was measured by taking double the volume as used for total phenol estimation, because the extract had been diluted two-fold and tannin-phenols are expected to be lost through binding with PVPP. The content of non-tannin phenols is expressed on a dry matter basis (y%) (Makkar 2003):

Tannins (as tannic acid equivalent) = x% – y% = Tannin% in the dry matter.

10. Rate of nitrification

The static incubation technique described by Pennington and Ellis (1993) was used. Initially, field moist soil equivalent to 10 g oven dry soil was placed in a 250-ml plastic bottle and top sealed with parafilm. Three replicates of each soil were kept in the dark at 20°C for one month. At the beginning and end of incubation, subsamples were extracted with 30 ml of 2N KCl and gravity filtered through pre-washed Whatman #42 filter paper. Extracts were analyzed for mineral N (NH_4^+ and NO_3^-). NH_4^+ content was determined using Bengtsson (1924) method and NO_3^- content determined using Devarda's alloy in alkaline solution as described in Jackson (1958).

Increases in NO_3-N and NH_4-N were recorded which determine nitrification and ammonification, respectively, which in turn reflect the relative activity levels of nitrifiers and ammonifiers (Pastor *et al.* 1984). The ratio of NO_3-N / NH_4-N was calculated as an indicative of nitrification and mineralization.

Soil microbiological assays

Soil samples were analyzed for predominant microbial populations namely bacteria, fungi and actinomycetes using appropriate selective growth media. The unsieved field moist soil samples were subjected to a microbial assay within 24 h of collection to minimize the effects of storage on microbial activity.

For the enumeration of active bacterial and actinomycetes population, a serial dilution method described by Dubey and Maheshwari (2002) was used. The soil samples were suitably diluted so that the number of colonies developing on the plate will fall in the range of 30-300 when the dilutions are cultured. A dilution blank (Ringer's solution - aqueous buffer containing 0.08% sodium chloride) was prepared. One gm portion of the soil was placed into a 99 ml of sterilized and cooled dilution blank. (Sterilization was done in autoclave at 15 lb/inch^2 for 30 min). This master suspension was a dilution of 10^{-2}. It was allowed to stand for 10-15 min, and then shaken one minute using a magnetic stirrer to get a homogeneous suspension for preparing additional dilutions. To dilute the samples quantitatively, a 1-ml aliquot from the master dilution was diluted stepwise through a series of tubes containing 9 ml of dilution blank, resulting in 10-fold dilutions. By continuing this dilution stepwise through additional dilution tubes, up to 10^{-9} dilutions were prepared. Before each transfer the tubes were mixed gently to get a uniform suspension. The soil samples were suitably diluted so that the number of colonies developing on the plate would fall in the range of 30-300 when the dilutions were cultured. Within this range the count could be accurate or else the numbers would become too low to count or too numerous to count. If discrete colonies were not growing in the medium their exact number could not be estimated.

Dilutions up to 10^{-9} dilutions were prepared (Robert 1967). Before each transfer the tubes were mixed gently to get a uniform suspension. L-rod (spread plate) inoculation technique was used for bacteria in sterile solid nutrient agar medium. Inoculation was done in 5 replicates. Incubation period was 24 h at 35°C. Pour plate inoculation method was used for enumerating actinomycetes in starch casein agar medium containing nystatin and actidione (Merck Co.). The soil sample was dried at 50°C for 15 min before the dilution process so that most bacteria and fungi could be killed. To facilitate the growth of actinomycetes, 2% $CaCO_3$ was mixed with soil. Five replication plates were prepared from each dilution. The number of bacterial and actinomycetes colonies formed was counted using an electronic colony counter. Actinomycetes colonies with white grey or black powdery surface having distinct halo with a darker interior and firmer colonies than bacteria were looked for. Microbial enumeration was done using the formula: Number of microbes per ml = Number of colonies counted on plate × dilution of sample. For fungal isolation, soil dilution plate technique was used (Warcup 1957). Martin's agar plates supplemented with both streptomycin and penicillin (30 mg/L, each) were used. Antibiotic addition in fungal medium prevented the growth of the bacterial and actionomycetes colonies. Antibiotics were added after autoclaving and cooling of the medium. The result was expressed as number of colony forming units (CFU) per ml. The colonies were counted and the average number of colony

forming units was calculated by using the following formula:

$$CFUs = \frac{\text{Average number of colonies} \times \text{Volume of sterile distilled water}}{\text{Dry weight of soil} \times \text{Dilution factor } (10^{-x})}$$

Statistical analysis

The data were analyzed using SPSS version 12 package. Means of five replications were taken for both physico-chemical and biological results The comparison of mean values were done using student's *t*-test and interpretation of effect size index for independent sample design were done as proposed by Cohen (1969). An effect size index is done for whether any significant difference should be considered small, medium or large. In the present study an effect size index of '0.5' and 'greater' are considered 'large'.

RESULTS

Soil physical and chemical assays

Both the test soils were dark grey in color. The tannin soil consisted of 48, 25 and 23 percent of sand, silt and clay respectively. The non-tannin soil consisted of 51.2, 27 and 21.8% of sand, silt and clay, respectively. The soils were medium textured sandy loam (alluvial sand). The dry matter, OM, C/N ratio, aggregate stability and nitrate contents were more in tannin soil when compared to non-tannin soil. Tannin soil had acidic pH. The EC was 2.9 dS/m for non tannin soil and 1.2 dS/m for tannin soil. An aggregate stability of 54 in non tannin soil and 52 in tannin soil was recorded in the study. The bulk density value and gravimetric water content was comparatively high in tannin soil. No tannin was detectable in non-tannin soil collected from the field (**Table 1**).

Soil microbes

Results of enumeration of predominant bacterial groups are given in **Table 2**. In non tannin soil, bacterial counts outnumbered fungal and actinomycetes counts ($P < 0.001$). As compared to other groups, fungi were the least in terms of abundance in non-tannin soils. The sequence of dominance in non tannin soil was bacteria< actinomycetes < fungi. The difference between the microbial counts of different groups within non-tannin soil was highly significant. The sequence of dominance in tannin soil followed the order fungi < actinomycetes < bacteria (but without statistical significance). In tannin soil, the counts of actinomycetes, bacteria and fungus did not differ significantly ($P > 0.05\%$). In tannin soil fungal counts predominated. Bacteria were the least among the identified groups of microbes in tannin soil.

DISCUSSION

Physical and biochemical properties of the soil

Statistical analysis of the data showed that there was no significant difference in the texture, pH, C/N ratio, bulk density and electrical conductivity of the two soils tested. Other physico-chemical properties of the soils showed that they did not differ much except for the highly significant difference in their tannin concentration, the effect size index being 0.8.The similar textural patterns of the test soils indicate that they have same degree of influence upon soil microbial growth. Their dark grey color may be due to the parental basaltic rocks. A slightly reduced pH of tannin soil may be due to the addition of acidic tannins by *A. nilotica* tree litter parts. Studies made by Nation (2007) and Ushio *et al.* (2010a) have shown that tannins reduce soil pH.

The tannin soil has higher C/N ratio when compared to non tannin soil. The decomposition rate is slower with higher C/N ratios and greater lignin content. Wider C/N ratios may indicate a N limitation for heterotrophic microbes, with greater potential for N immobilization (Balota and Chaves 2010). The mineralization ratio is highly influenced by other residues characteristics, such as polyphenolics, lignin and cellulose, as well the relationship between them (Tian *et al*. 1992). The plant's polyphenol content can affect the residue decomposition because polyphenols may form a complex with proteins, thus reducing N availability to microorganisms (Monteiro *et al*. 2002). A wider C/N ratio of tannin soil in the present study indicated a slower microbial litter degradation and nitrogen mineralization rate which may be due to the presence of phenolic inhibitors i.e., tannins. These changes alter the potential for the soil to supply or to sequester nutrients due to changes in mineralization and immobilization (Franzlubbers *et al*. 1995). Therefore, residue decomposition is an important driving variable in the nutrient cycling processes. Litter decomposition might be slowed down if the protein-binding properties of tannins affect functioning of extracellular fungal enzymes (Lorenz and Preston 2000). Phenolic compounds, especially tannins, have been shown to affect soil C and N transformations; they complex with proteins and possibly other N-containing compounds, metal ions and other

Table 1 Physico-chemical properties of soils in study sites.

Parameter	Non-tannin soil	Tannin soil	Effect size Index
Dry matter (%)	62.5 ± 0.65	85.0 ± 0.45*	0.16
Organic matter (%)	4.00 ± 0.762	14.0 ± 1.34*	0.31
Carbon (%)	2.32 ± 1.25	7.71 ± 1.62*	0.4
Total nitrogen (%)	0.07 ± 0.008	0.21 ± 0.012*	0.002
C/N ratio	33.1 ± 1.21	38.5 ± 4.82^{NS}	-
pH	7.40 ± 0.376	6.61 ± 0.592^{NS}	-
Electrical conductivity (dS/ m)	2.90 ± 0.965	1.20 ± 0.985^{NS}	-
Aggregate stability (%)	54.0 ± 1.98	49.0 ± 1.55*	0.5
Bulk density (g cm^{-3})	1.05 ± 0.08	1.25 ± 0.09*	0.03
Gravimetric water content (g g^{-1} dry soil)	0.64 ± 0.065	0.73 ± 0.002*	0.01
Total tannins (%)	0.006 ± 2.41	5.02 ± 3.24*	0.8
Nitrate nitrogen (µg g^{-1} dry soil)	33.0 ± 2.53	4.00 ± 0.275*	0.5

Values are mean of five replicates with SEM values given in parenthesis.
*Values within a row differ significantly *($p<0.05$).
^{NS} Values within a row do not differ significantly.
Effect size index: Small = 0.2, Medium = 0.5, Large = 0.8 (Cohen, 1969)

Table 2 Viable population size of predominant microbes in two soil types.

Microbes	Non-tannin soil[a]	Tannin soil^{NS}	SED
Bacteria (CFU × 10^4/g)	720 (0.045409)	5.5 (0.067305)	0.081191**
Fungi (CFU × 10^4/g)	5.8 (0.048926)	5.7 (0.048156)	0.06865
Actinomycetes (CFU × 10^4/g)	65 (0.063241)	5.6 (0.063889)	0.089895**

*($p<0.001$), n=5. SEM values given in parenthesis
[a] Microbial groups differ significantly within columns at p< .01 level
^{NS} Microbial groups do not differ significantly

macromolecules like polysaccharides, induce toxicity to microbes and inhibit enzyme activities in soil (Fierer *et al*. 2001, reviewed by Schofield *et al*. 2001; Kraus *et al*. 2004a). Nierop *et al*. (2006a) from her studies found that net N mineralisation in soil decreased due to the addition of condensed tannins.

Tannins are large complex organic molecules resistant to microbial degradation. High molecular weight condensed tannins were resistant to degradation and were microbial toxic affecting the nitrogen cycling in the forest floor (Kanerva 2007). Slower decomposition rate in tannin soil must have accounted for higher organic carbon content in it. The accumulation of undegraded organic matter and slow decomposition in black spruce forests are favored by tannins in needle litter (Waring and Schlesinger 1985). Through formation of protein-tannin complexes, nitrogen mineralization may be inhibited and nitrogen cycling may shift from a mineral to organic dominated pathway. Norway spruce (*Picea abies* (L.) Karst), rich in tannin has been found to change soil fertility gradually in an unfavorable direction by lowering the soil pH, decomposition rates and concentration of exchangeable nutrients, by increasing soil C-to-N ratio and by enhancing podsolisation (Priha and Smolander 2000; Menyailo *et al*. 2002).

Tannins in this way may help to preserve the organic matter content of the soil. Many forest plants growing in tannin-rich soil environments develop biological systems to circumvent the need for exogenous N-mineralization pathways. Plants may compensate for the slow rates of nutrient cycling, associated with litter containing large amounts of tannins, by increasing the production of fine roots (Fischer *et al*. 2006) and symbiotic association with tannin degrading ectomycorrhizae (Read *et al*. 2004). Trees with such abilities may win competitive advantage to thrive well and establish a stand in tannin rich forest floor. Similarly, among the microbes too there exists competition for existence and dominance in tannin rich forest floor.

Soil microbiological properties

A comparatively higher nitrate content of 33 µg/g dry non tannin soil against 4 µg/g dry tannin soil suggests that nitrate producing nitrifiers are densely populated in non tannin soil in the absence of tannin inhibitor. In tannin soil, as the organic nitrogen breakdown was slowed, there was little ammonia production and nitrifiers which influence ammonia conversion to nitrate were retarded by the presence of tannin. Tannin's greatest impacts are seen in the leaf litter and soil where they have been reported to inhibit the activity of fungi (Harrison 1971) and nitrifying bacteria (Rice and Pancholy 1973), their increased number reduces soil ammonia content.

A higher microbial load of non tannin soil when compared to tannin soil could have contributed for a higher aggregate stability and lower bulk density values. Villar *et al*. (2004), from their studies related to soil rehabilitation, had reported a high positive and significant relationship between soil microbial biomass and aggregate stability. Soil microorganisms process plant litter and residues into soil organic matter, which improves soil quality by increasing soil aggregation and aeration and decreasing soil bulk density (Dominy and Haynes 2002; Spaccini 2002). Microbes can help to stabilize the soil by physically binding soil particles together by releasing by-products that acts as a "glue" to help bind clay particles and organic materials together to contribute to soil aggregation (Lu and Pignatello 2002).

Total microbial counts in non tannin soil were relatively higher than the tannin soil but were found lower than the normal soil counts. The bacterial, actinomycete and fungal counts in a fertile soil are in the range of 10^8–10^9, 10^7–10^8 and 10^5 to 10^6/g soil, respectively (Ashton and Macintosh 2002; Delgado and Follett 2002). The lower counts of microbes in non tannin soil than normal may be because of the low content of organic matter and low water holding capacity of the soil when compared to a fertile soil. Also nitrogen content was low due to depletion by excessive mineralization. The lower organic matter content of non tannin soil must be due to intensive cropping that decreases humus content gradually as suggested by Lal (1999) which may cause rapid deterioration of soil physical properties.

Though tannin soil had much organic matter, the level of organic matter or nitrogen reflects more a potential than an actual supply. Soil quality should be determined in terms of microbial activity rather than considering the organic matter content. Soil organisms metabolize organic pollutants heavy metals, excess nutrients or immobilize them in their biomass and necromass (Diplock *et al*. 2009), thereby incorporating them into stable humus (Moeckel *et al*. 2008). Humus improves the physical properties of the soil and thus its fertility. The physical integrity of soil is also a prerequisite for avoiding landslides in rugged landscapes (Rezaei *et al*. 2009). The size and activity of the soil microbial population influence the rate of decomposition (Parnas 1975). The undegradable type of organic matter does not support microbial population. Many microbial enzymes get complexed with tannins and often exhibit reduced activity (Goldstein and Swain 1965).

Usually bacteria predominate in any soil system the least being fungi (Ashton and Macintosh 2002; Delgado and Follett 2002). Actinomycetes fare poorly in competition with bacteria during the period when simple carbohydrates or usual carbon and nitrogen mineralization were present. In tannin soil fungi predominated unlike the bacterial predominance in natural condition. Tannins in soil must have suppressed the bacterial growth. Acidic tannins of TS must have favoured acidophilic fungi. The fungi on the other hand must have developed the ability to degrade acidic tannins and by the way of degradation must have added to soil acidity further. The lowered pH is not a suitable environment for Actinomycetes and bacteria which prefer slightly alkaline or neutral pH respectively. The forms flourishing in any particular microenvironment are the ones best adapted to the environmental conditions (McLean and Parkinson 1997).

It was also found that within tannin soil the Actinomycetes counts were found higher than bacterial counts (**Table 2**). Suppression of bacterial competitors must have allowed actinomycetes to thrive well in slightly acidic soil. Actinomycetes are associated with microbial antagonism and regulation of composition of the soil community. Many Actinomycetes can excrete antibiotics or have the capacity to produce enzymes that are responsible for lysis of fungi and bacteria. Actinomycetes are usually effective competitors only when resistant compounds remain (Walksman 1967). Thus tannin soil favoured fungi and actinomycetes more than the bacteria.

CONCLUSION

As the tannins enter the soil organic matter pool, they may affect several aspects of ecosystem functioning. It had been noted that in non tannin soil, more plant growth favorable physico chemical properties were observed which could be mainly due to a higher microbial load when compared to the tannin soil. In tannin soil, since allelochemical - tannins inhibit microbial activities, a lower count and distribution of microbes was found. In acidic tannin soil fungi predominated unlike the bacterial predominance in natural condition. As tannins inhibit nitrifier populations in soil, there was no much detection of nitrates and thus nitrogen losses from leaching and denitrification are greatly attenuated. Though considered to be an allelochemical, future researches can be focused in this aspect of tannin's ability to retain soil organic matter and save soil nitrogen. The allelochemical nature of tannins may be exploited for weed growth control too.

ACKNOWLEDGEMENTS

We greatly acknowledge Prof. Dr. C.K. Singh, Department of Animal Nutrition, Veterinary College, Karnataka Veterinary, Animal and Fisheries Sciences University, Bangalore-4, for having helped us by providing lab facilities to carry out chemical analytical works related to the present study.

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