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Assessment of litter degradation in medicinal plants subjected to ultraviolet-B radiation

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Abstract

Litter decomposition is an important component of global carbon budget. Elevated influx of ultraviolet-B radiation (UV-B) as a consequence of depletion of stratospheric ozone (O_3) layer may affect litter decomposition directly or/ modifying the plant tissue quality. Chemical composition of plant can affect litter decomposition. In the present study, three important medicinal plant species *i.e. Acorus calamus, Ocimum sanctum and Cymbopogon citratus* were exposed to two levels of supplemental UV-B (sUV_1 and sUV_2) during the growth period and examined the changes in leaf quality and degradation of leaf litters. The sUV_2 treatment (+3.6 kJ m^{-2} d^{-1}) increased the rate of decomposition by 45% and 31% respectively; in leaf litters from *O. sanctum* and *C. citratus*, while no significant effect was noticed in *A. calamus* leaf litter. Higher accumulation of sclerenchymatous tissue around vascular bundles and increased concentrations of total phenols by 39 mg g^{-1} probably lowered the decomposition rate; finding k value: 0.0049 g g^{-1} d^{-1} in leaf litters of *A. calamus*. The C/ N ratio was increased by 14% at sUV_2 in *C. citratus*, whereas in *O. sanctum* it decreased by 13.6% after treatment. Results of the present experiment illustrates that firstly UV-B can modify the decomposition rate of leaf litter of test plant species, secondly it can alter the tissue chemistry particularly leaf phenolics, N and P concentrations strongly and thus affecting the decay rate and thirdly UV-B effects on decay rate and leaf chemistry is species specific.

Key words

Ultraviolet-B, Medicinal plants, C/N ratio, Decomposition rate

Introduction

Ultraviolet radiation is an important component of the sunlight reaching the surface of the earth and only 0.5% of this consists of biologically effective UV-B radiation (Caldwell *et al.*, 1982), which can affect various ecological processes such as decomposition processes (Zepp *et al.*, 2007). Litter decomposition plays a key role in determining nutrients availability and storage of organic C reservoir in terrestrial ecosystem and any change in this process will have severe consequences on the structure and function of ecosystem. Litter decomposition and various factors controlling this process are important for studying nutrient cycling, and developing C budgets with the relative implications in the global climate change. Leaf chemistry (e.g. lignin and phenol contents, C/N ratios) and abiotic stress variables such as temperature, precipitation and solar radiation can influence litter decomposition rate at both regional and global scales (Brandt *et al.*, 2007; Siegenthaler *et al.*, 2010; Zepp *et al.*, 2007). Leaf litter decomposability is also related to the physical properties of living leaf tissues such as leaf toughness (Kazakou *et al.*, 2006). Solar radiation especially in the ultraviolet (UV-B) range (280– 400 nm), may play a role in the decomposition through photodegradation (Austin and Ballare, 2010).

Several studies have shown that photodegradation can affect the decomposition of organic matter by either directly mineralizing organic carbon or by facilitating microbial decomposition, because some of the photodegraded plant components physically prevented microbial degradation. In addition, UV-B exposure may affect decomposition indirectly by modifying the plant secondary metabolism thus modifying leaf tissue chemistry (Brandt *et al.,* 2007; Gehrke *et al.,* 1995; Rozema *et al.,* 1997). For example, UV-B exposure can shift C allocation to phenylpropanoid pathway, which produces specific secondary carbon compounds. Complex carbon compounds such as polyphenols can retard litter decomposition and nutrient mineralization in soil as these compounds are quite resistant to microbial breakdown and thus the release of carbon to the atmosphere is decreased (Duguay and Klironomos, 2000; Pancotto *et al.,* 2005).

The importance of leaf quality parameters such as C/N ratio and chemical composition on litter decomposition have been extensively studied in mesic ecosystems (Aerts and de Caluwe, 1997; Wardle, 2002). Sclerenchymatous tissue hardening can influence the quality of the litter produced by providing mechanical strength to the plant tissues and can retard litter decomposition.

Three medicinal plants of *Acorus calamus, Ocimum sanctum* and *Cymbopogon citratus*; rich in medicinal property (potential source of drugs) and essential oils of therapeutic importance were selected as test plants. The rhizomes and leafy parts of *A. calamus* possess anti-spasmodic, carminative and anthelmintic hypotensive and antidepressant properties (Raina *et al.,* 2003). Several studies reported its antiproliferative, immunosuppressive and anti-carcinogenetic activity on human carcinoma cells (Kumari *et al.,* 2009; 2010). *O. tenuiflorum* (holy basil), ranks among the most important aromatic shrub in Ayurvedic medicine having anti-cancerous, anti-diabetic, spasmolytic, carminative, cardioprotective, anthelmintic and diaphoretics properties (Kumari and Agrawal, 2011). *C. citratus* (lemongrass), belongs to the family Poaceae, is a perennial grass, and is highly recommended for bacterial and fungal infections, to treat digestive disorders, diabetes, nervous disorders, fever as well as other stress related health problems. The tea prepared from leaves of lemongrass has been used as a nerve tonic (Cheel *et al.,* 2005).

In light of the above, the objectives of this study were to examine the long-term (period) effects of UV-B exposure (sUV-B) on nutrient contents and litter chemistry of *A. calamus, O. sanctum, C. citratus* and to investigate whether changes in tissue quality due to UV-B exposure was responsible for change in the rate of litter decomposition.

Materials and Methods

Plant material : Rhizomatous/bulbous stems of sweet flag (*Acorus calamus*) and lemongrass (*Cymbopogon citratus*) and seeds of holy basil (*Ocimum tenuiflorum*) were collected from the Department of Horticultural Science, Institute of Agricultural Sciences, BHU, Varanasi, and transplanted in experimental plots by conventional methods. Plants were watered regularly and uniformly to maintain optimum water regime in each plot.

Experimental set-ups and sUV-B treatment : The experimental plan included three treatments: i) ambient UV-B (control) and ii) supplemental UV-B exposure at sUV$_1$ (±1.8 kJ m^{-2} d^{-1}) and ii) at sUV$_2$ (±3.6 kJ m^{-2} d^{-1}). Nine plots (three replicates for each treatment) were randomly designed for experiment. Each plot had an area of 1m × 1 m. No fertilizer was added during the experiment.

The UV-B exposure was provided artificially by 'Q Panel UV-B 313, 40 W fluorescent lamps' (Q Panel Inc., Cleveland, OH, USA) and plants were irradiated for 3 hr per day (10.00–13.00 hr) up to 100 days after transplantation. Cellulose diacetate and polyester films of 0.13 mm thickness were used to transmit UV-B (cut off ca. 292 nm) and exclude UV-B (cut off ca. 318 nm), respectively for the UV-B and control treatments. The plastic sheet was also inter-positioned vertically to avoid lateral transmission of light between adjacent plots. These filters were changed weekly (at 7 days) to avoid the photo degradation effect caused by prolonged UV-B treatment.

The UV-B irradiance at the top of the plant canopy was measured after 100 days with Ultraviolet Intensity Meter (Model UVP Inc. San Gabriel, (A), USA). The readings were converted to biologically effective (UV-BBE) values by the Spectro-Power Meter (Model Scientech, Boulder, USA). Control plants under the polyester film only received ambient UV-B (9.6 kJ m^{-2} UV-B$_{BE}$) on the summer solstice weighted against generalized plant response action spectrum (Caldwell, 1971). In the sUV-B treatment set ups, UV-313 fluorescent lamps were covered by 0.13 mm thick cellulose acetate filters (transmission down to 280 nm) to transmit UV-B radiation. The plants beneath the cellulose diacetate film receiving ambient + supplemental sUV$_1$ and sUV$_2$ doses (+1.8 and +3.6 kJ m^{-2}) that mimicked the level of 5 and 10% reduction in stratospheric ozone layer; at Varanasi during clear sky condition (Green *et al.,* 1980).

At planting, soil was initially sampled at a depth of 0–0.3 m, air-dried, passed through a 2-mm sieve and analyzed for organic carbon (Walkey and Blac, 1934), total nitrogen (micro-Kjeldahl technique) and available phosphorus content (Olsen *et al.,* 1954).

Plants in three replicate subplots of each treatment were harvested from at 100 days after UV-B radiation (physiological maturity). Nitrogen content in leaf was determined by the Kjeldahl method (Bremner and Keeney, 1965). Organic carbon content in leaf was determined by modified oxidation method (Walkley and Black, 1934).

Total phenol was determined with Folin–Ciocalteuo reagent following the method of Bray and Thorpe (1954) and quantified by spectrophotometric analysis at 650 nm, using a UV–vis spectrophotometer (Model, 119, Systronics India).

Transverse sections of fresh leaf tissue from both control and sUV-B treated plants, taken at a point approximately one-third of the lamina length from the tip of leaves were cut (1.0 mm thick) and mounted on gelatin-coated slides. Sclerenchymatous tissues around the vascular bundles were visualized by staining the leaf sections in phloroglucinol reagent for 30 min at room temperature. Stained sections were than examined under a light microscope (Olympus BX51) at 1000 × magnifications using transmitted light.

Leaf decomposition study was carried out from April to June, 2007 by the litter-bag technique (Osono and Takeda, 2001). Litter-bag (10 × 10 cm) were made by sewing UV-B stabilized polyester net of 2 mm size and were filled with dried and chopped leaf litters (1.0 cm) prepared from control and sUV-B treated plants (5 g dry weight). Litter-bags were buried 5 cm deep in soil and sampled periodically to estimate decomposition. Each treatment was replicated six times and bags were placed in the field plots in a randomly block design. For biomass assay, retrieved litterbags were examined at time of sampling on site and any extraneous materials (e.g., sand, stone, etc) were removed from litter. Samples of each were weighed after drying in an oven at 60°C for 48 h to determine the constant weight. Dry weight of litter remaining (%MR) was calculated and annual fractional weight loss (decay rate; $g\ g^{-1}\ d^{-1}$) of leaf litter was estimated.

Residual dry weight of litter mass was determined after 30, 60 and 90 days, then percentages of total litter undecomposed was calculated at each time and the annual rate of decomposition constant (k) of leaf litter was estimated from these data by negative exponential decomposition model proposed by Olson (1963).

One-way ANOVA analyses followed by Duncan's test were used to evaluate significant differences. Bivariate ANOVA test was applied to identify the significant effects. All statistical tests were performed with SPSS software (SPSS Inc., version 10.0, Chicago, Illinois, USA).

Results and Discussion

Ultraviolet-B effects on litter decomposition : The UV-B applied during the growth period in plants accelerated the mass loss and decomposition of leaf litter processes in *O. sanctum* and *C. citratus* in soil. This effect was more consistent and greater in case of the *O. sanctum* than *C. citratus* leaf litter. The data on percentage mass retained after decomposition of leaf litters of different test plants are shown in Fig.1. Highest mass loss as well as k values (values of decomposition constant) was observed in the leaf litter of O. *sanctum* followed by *C. citratus* (Fig.1, 2). Time (decomposition period) had significant effect on litter decay rate in *O. sanctum*. After 30 days of decomposition, the loss percentage of litter mass decreased by 11 and 16% after irradiation with UV$_1$ and UV$_2$ respectively, as compared to control plants with increases in k values of litters. This response was substantially greater when observed at 60 and 90 days than at shorter times and showed an increase of 40 and 45% in the k value with sUV$_2$, respectively. ANOVA test showed the significant variations due to time and treatment and also due to interaction of decomposition time (A) × treatment (T) in *O. sanctum* (Table 1). Similar trend was also observed in *C. citratus* and k values were increased by 11.2 and 31% after the 90 days of decomposition period with sUV$_1$ and sUV$_2$, respectively (Fig.2). ANOVA test also indicated that value of k and percentage of litter mass significantly varied with treatment in *C. citratus*. Contrary to this, no significant effect of UV-B was noticed on litter decomposition in *A. calamus,* evaluated as either the percentage of mass loss or its decomposition constants (k-value) ($p<0.05$) (Fig, 1 and 2). Bivariate analysis (Two Way ANOVA) also revealed that there was no significant treatment effect of UV-B or interaction of age (A) × UV-B on both parameters (Table 1) in *A. calamus*.

Probably the difference in rate of litter decomposition was mainly due to the photodegradation or the physiochemical transformation of decomposing compounds into smaller compounds due to high energy photons rather than an increased microbial activity (Austin and Vivanco 2006; Austin and Ballare 2010; Brandt *et al.,* 2007; Pancotto *et al.,* 2005). Day *et al.* (2007) reported that about 14-22% of the total mass loss of *Larrea tridentate* leaf litter after 4-5 month was mainly due to loss of lignin, fats and lipids. Rozema *et al.* (1997) showed increased mass loss rates of

Table 1 : Level of significance of litter decomposition constant (k) and % mass remaining in different medicinal plants after exposure with UV-B

Plant	**Treatment**	**% Mass remaining**	**Litter decomposition constant (k)**
A. calamus	Time (A)	***	**
	Treatment (T)	ns	ns
	A×T	ns	ns
O. sanctum	Time (A)	***	***
	Treatment (T)	***	***
	A×T	*	*
C. citratus	Time (A)	***	***
	Treatment (T)	*	**
	A×T	*	*

Level of significance:*$p<0.05$,**$p<0.01$,***$p<0.001$,^{ns}:not significant

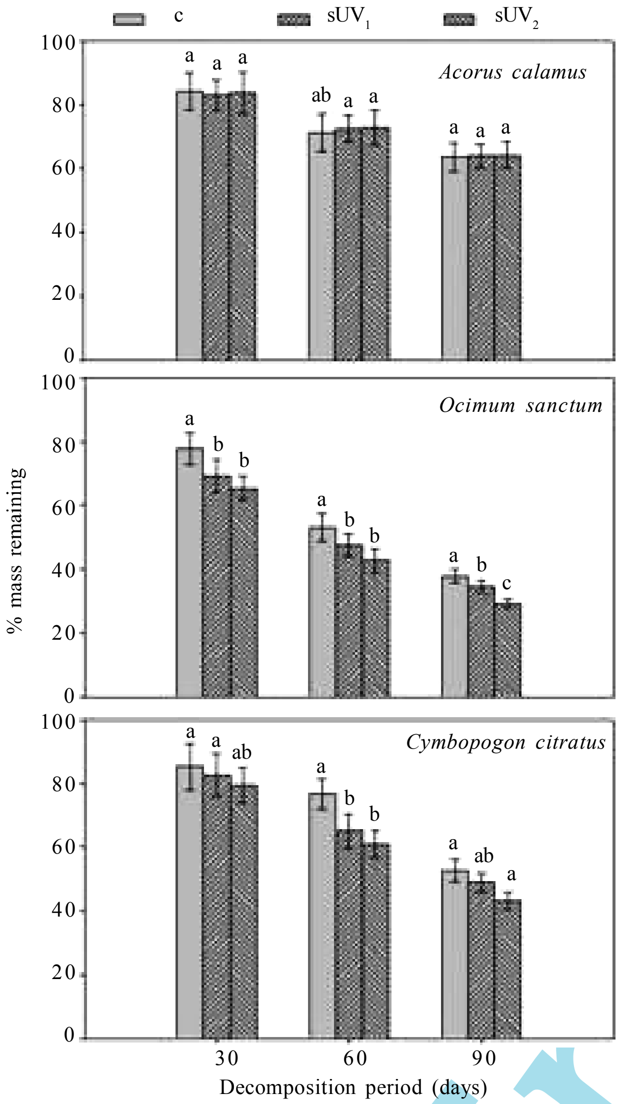


Fig. 1 : Values of % mass remaining for leaf litters from *A. calamus, O. sanctum* and *C. citratus* at different period over 90 days exposure of UV-B. Bars followed by the different letter within same group indicate statistically significant difference by Duncan's test at p<0.005 (mean ± SE (p< 0.05). C is control, UV_1 is lower dose of UV-B, UV_2 is higher dose of UV-B

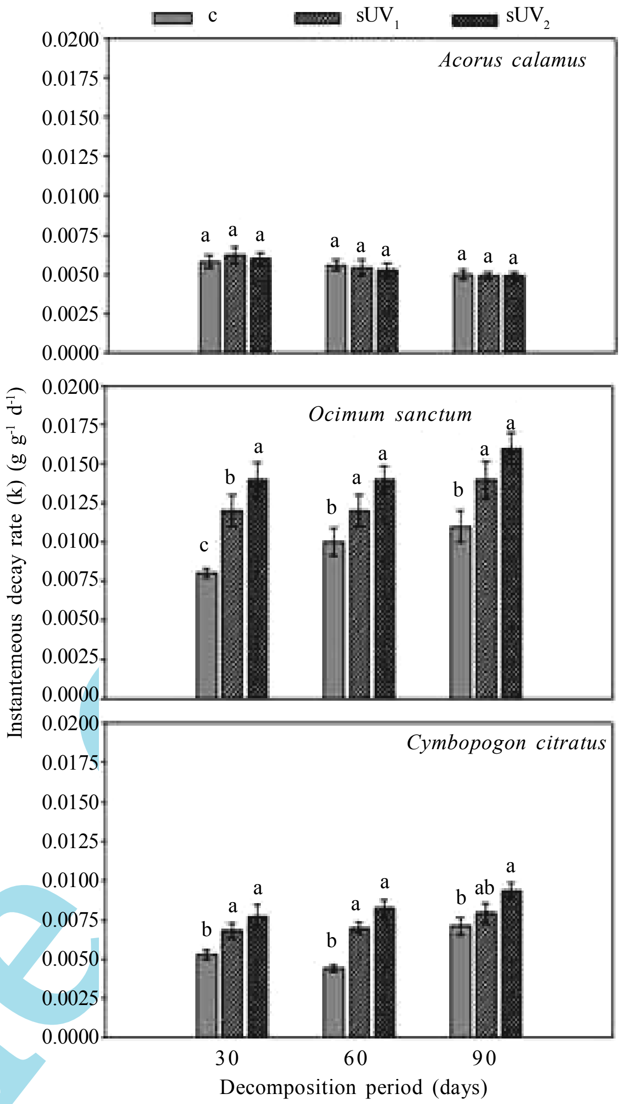
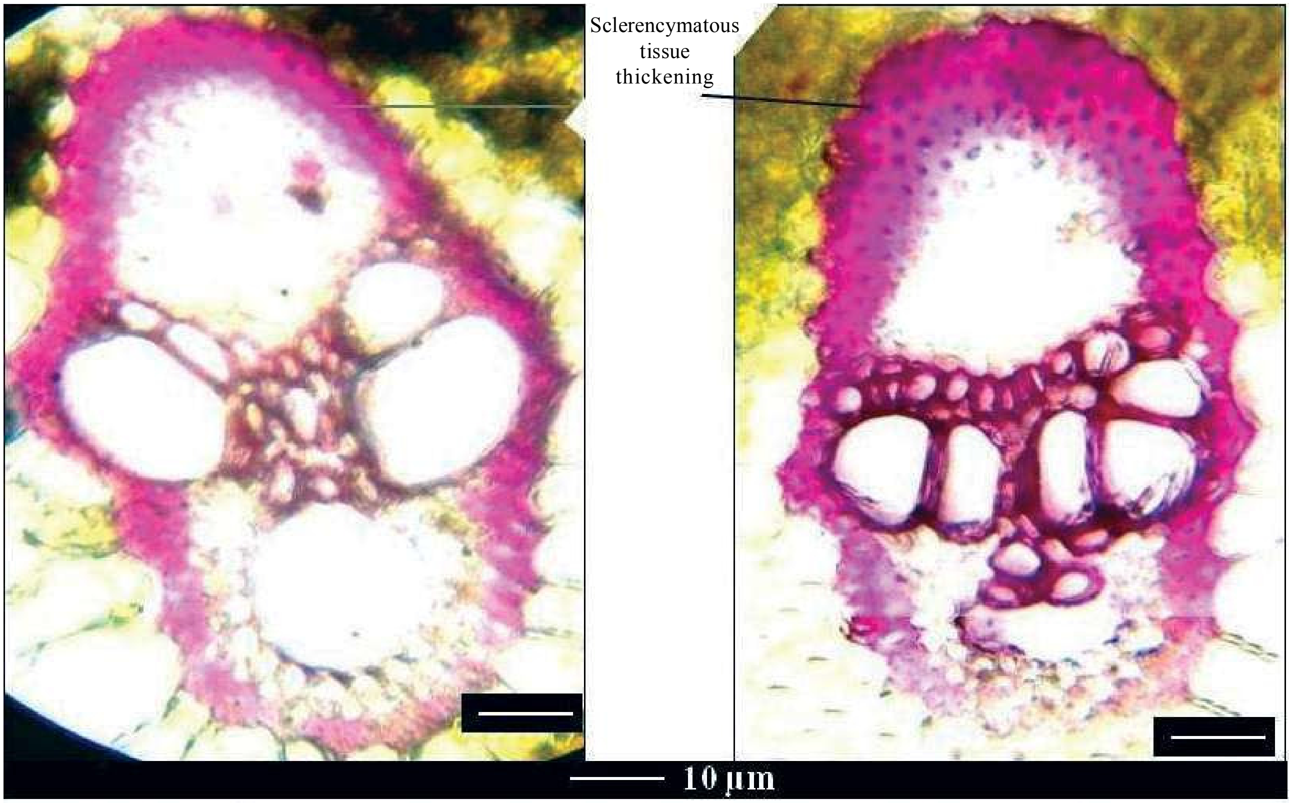


Fig. 2 : Instantaneous decay rate (k values) for leaf litters from *A. calamus*, *O. sanctum* and *C. citratus* at different period over 90 days exposure of UV-B. Bars followed by the different letter within same group indicate statistically significant difference by Duncan's test at p<0.005 (mean ± SE (p< 0.05). C is control, UV1 is lower dose of UV-B, UV2 is higher dose of UV-B

Calamagrostis epigeios with lower concentrations of lignin and hemicellulose in the residual litter subjected to increased UV-B (approx. 33% more than ambient). The significant increase in decay of litters of *O. sanctum* and *C. citratus* confirm the earlier studies on loblolly pine (Cybulski *et al.*, 2000), *Quercus robur* (Newsham *et al.,* 1999) and *Larrea tridentate* (Day *et al.,* 2007) litter. Using litterbags, Rozema et al. (1997) found that increased UV-B exposure increased the decomposition rate of *Calamagrostis epigeios* litter by 5–10% in a dune grassland. On the other hand, in the present study, the sUV-B did not affect the mass loss and decomposition constant in *A. calamus* and confirms earlier studies showing insignificant or only marginally significant effects of UV-B on rate of litter mass loss (Hoorens *et al.,* 2004). Verhoef *et al.* (2000) observed no difference in the mass loss rates of the dune grasses, *Calamagrotis epigeios* and *Carex arenaria*, under elevated UV-B. Several studies have found that elevated UV-B either did not affect decomposition or reduced decomposition of plant litters because of its effects on the microbial community (Duguay and Klironomos, 2000; Gehrke *et al.,* 1995; Newsham *et al.,* 1997). However, no general conclusions can be drawn about the UV-B effects on litter decomposition on the basis of studies made so far.



Control sUV-B

Fig. 3 : Transverse sectional of leaf showing comparative change in leaf tissue thickening around vascular bundle in control and UV-B exposed A. *calamus* plants

A possible explanation for this inconsistent trend of decay rate obtained in the test plants may be the due to variation in their growth form and species type (*A. calamus*: herb; *O. sanctum*: shrub and *C. citratus*: perennial grass). Climate, soil or plant growth forms are thought to be the primary factors influencing the leaf chemistry such as N and P concentration, which linked with decomposition processes (Chen *et al.,* 2011). In global meta- analysis of variation in leaf litter quality and decomposition among 818 species demonstrates that plant growth form can have strong effects on decomposition and soil processes (Cornwell *et al.,* 2008). In aspect of UV-B, it has been speculated that many chemicals important to decomposition may be altered by UV-B induced changes in the general phenylpropanoid pathway (Cybulski *et al.,* 2003). Of significance, UV-B induced modifications in tissue chemistry are strong determinate to change the litter decay decomposition.

Ultraviolet-B effects on leaf tissue chemistry/ quality : The UV-B treatment applied during plant growth changed leaf tissue chemistry and litter quality. In *A. calamus* anatomical observation showed structural changes in vascular tissues as observed by hand cut sections of leaves after UV-B treatment. The treatment with sUV-B developed higher thickening of sclerenchymatous tissue in hypodermal region around vascular bundles (Fig.3). In *O. sanatum* and C. *citratus*, slight increase in thickening of epidermal cuticle was observed after UV-B exposure.

Leaf nutrient concentration plays an important role in the decomposition of litter in most arid and semi-arid ecosystem (Brandt *et al.,* 2007). In present experiment, initially, C/N ratio was lowest for *O. sanctum*, intermediate for *A. calamus* and highest for *C. citratus*. Leaf litter of *O. sanctum* with the lowest C/ N ratio is expected to decompose more readily and indeed it occurred. After exposure with UV-B, *O. sanctum* showed an increase in the concentrations of N and C by 29 and 16% respectively under sUV_2 than their respective controls. Whereas C: N ratio decreased by 15 and 18% respectively under sUV_1 and sUV_2 than their respective controls. The N content of *O. sanctum* leaves increased ($P<.05$) more than the C content by UV-B treatment and so C/N ratio declined. On the other hand, in *C. citratus*, C/N ratio increased by 14 and 28% in sUV_1 and sUV_2, respectively as compared to their controls (Table 2). The C/ N ratio was not changed by UV-B in *A. calamus*. Usually the rate of decomposition is increased by decreasing the C/ N ratio as the N content is commonly considered an important parameter controlling the rate of decomposition

Table 2 : Species specific differences in initial litter chemistry of control and sUV-B (sUV$_1$ and sUV$_2$) treated leaves of *A. calamus*, *O. sanctum* and *C. citratus*

Plants	Treatment	Leaf nitrogen content (mg g^{-1} f. wt.)	Leaf carbon content (mg g^{-1} f. wt.)	C:N	Leaf phenolic content (mg g^{-1} f. wt.)
A. calamus	C	24.8± 2.1^{a}	479.4±22.6^{a}	18.97±0.9^{ab}	38.7±1.4^{a}
	sUV$_1$	22.4 ± 2.6^{a}	448.7±19.7^{b}	20.45±1.1^{a}	36.4±2.2^{a}
	sUV$_2$	23.2 ± 3.1^{a}	487.6±30.1^{b}	22.08±1.5^{a}	39.1±2.1^{a}
O. sanctum	C	32.7 ± 2.4^{b}	463.7±19.7^{a}	14.34±0.7^{a}	19.8±1.1^{b}
	sUV$_1$	40.5± 3.6^{a}	529.8±15.4^{b}	12.88±1.2^{b}	21.3±1.9^{a}
	sUV$_2$	42.2 ±2.5^{a}	537.5±16.3^{b}	12.39±1.7b	24.4±1.6^{a}
C. citratus	C	28.5±2.2^{a}	627.2±33.6^{a}	23.5±2.0^{b}	20.5±2.6^{b}
	sUV$_1$	27.3±2.6^{a}	689.7±29.5^{a}	24.89±1.7^{b}	24.7±1.7^{a}
	sUV$_2$	25.2±1.8^{a}	678.5±36.7^{a}	26.78±2.3^{a}	26.3±1.8^{a}

Results are mean of three replicates ± S.E. . C is control, sUV$_1$ is lower dose sUV-B, and sUV$_2$ is higher dose sUV-B; Values with different letters in the same column differ significantly at $p<0.05$

(Melillo *et al.,* 1982). Zaller *et al.* (2009) showed the decline of C/N ratio under high UV-B treatments in a field experiment on *Carex* fen ecosystem. This corresponded well with our results showing lower value for C/N for *O. sanctum* leaves under UV-B as compared to control, which decomposed at faster rate. It is also important to note the increment in the decomposition rate of leaf litters of *C. citratus* after UV-B exposure despite their high C/N ratio. Probably the increase in the decomposition rate due to the UV-B effect on *C. citratus* might be due to the increase in the photodegradation.

Total phenolic content was found to be highest in leaf litter of *A. calamus* as compared to *O. sanctum* and *C. citratus*. Upon treatment, total phenol found to be increased by 23% in *O. sanctum* and 28% in *C. citratus* under higher dose sUV$_2$ in respect to control, whereas it was unchanged by UV-B in *A. calamus* (Table 2). The decay rates of litters from various plant species is related to the concentration of total soluble phenols (Valachovic *et al.,* 2004). It was proposed that the higher concentrations of phenols in plant litter might reduce microbial activity, with a resulting decrease in decomposition rate (Gehrke *et al.,* 1995; Rozema *et al.,* 1997). Phenolic compounds may directly affect the decomposition and activity of decomposers community, thus influencing the rate of decomposition (Hattenschwiler and Vitousek, 2000). In the present study, the maximum content of total phenolics found in *A. calamus* litter which showed the minimum rate of decomposition. Phenols have been shown to inhibit the digestion of cell walls, proteins, and carbohydrate by soil microbes (Van Soest, 1994). They can inhibit microbial enzymes to be toxic on decomposers (Siegenthaler *et al.,* 2010). Phenol polymers provides mechanical rigidity to plant cells and tissues, thus inhibiting their decomposition and can also form resistant complexes with proteins (Hattenschwiler and Vitousek, 2000), protecting them against degradation. Phenolics are strong UV absorbers (Day *et al.,* 2007). This may explain the slower decomposition rate in *A. calamus* than other plants since its concentration of phenolics increased non-significantly after sUV-B exposure as compared to *O. sanctum* and *C. citratus*. Hattenschwiler and Jorgensen (2010) reported that the increase of total phenolic concentrations in tropical vegetation increase the resistance of litter to decompose by inhibiting microbial breakdown/ photodegradation. The results of this study support the hypothesis that exposure to elevated levels of UV-B can alter the chemical composition and decay rate of plant litter.

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