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Inhibitory Effect of Leaf Extract and Leaf Mulch from Selected Tree Species on Physiology of Millet Under Nursery Condition

Sale, F.A[1] and Oyun, M.B[2]
1. Department Of Forestry And Wildlife
Faculty of Agriculture, Kogi State University, Anyigba, P.M.B 1008, Kogi State, Nigeria
E-Mail: Faithoguche@Yahoo.Com
2. Department Of Forestry And Wood Technology,
Federal University of Technology, Akure, P.M.B 704, Ondo State, Nigeria

Abstract
Inhibition of plant residues may release secondary metabolites that can favourably or adversely affects other plants. The overall objective of these study is to determine whether the leaf extract and decomposition products of leaf mulch of *Acacia auriculiformis, Eucalyptus citriodora* and *Gliricidia sepium* contain allelochemicals that are inhibitory to the physiology of millet (*Panicum miliaceum L.*). In a pot experiment, application of leaf extract depressed the dry weight of millet seedlings relative to the control with the observed phytotoxic interference not increasing with extract concentration except for *Eucalyptus* and *Gliricidia* extracts. The results also showed that in terms of duration, the inhibitory effect waned with time and was more pronounced at two weeks after application than at six weeks. It is evident from the results that the severity and persistence of the inhibitory effect of the extracts was in the order: *Gliricidia* > *Eucalyptus* > *Acacia.* The results of pot experiment also showed that there were difference between extract and mulch in their inhibitory effects as phytotoxic interference occurred earlier in pots that received extract solution compared to mulched pots. In contrast to results obtained when extracts were added, high rates of mulch application did not interfere to reduce dry weight but increased it relative to control. The present results demonstrate appreciable and varying degree of phytotoxity exhibited by the mulch species of millet plant through inhibition of its physiological processes such as seed germination, chlorophyll formation and growth.
Keywords: Inhibition, Tree species, Physiology and Millet Plant

Introduction

The concept of inhibition is still a matter of controversy (Aldric, 1994) and is plaqued with methodological problems, particularly those of the distinguishing effects of allelopathy from competition (Willis, 1985). Only a few investigations have separated the components of interference because of the complexity of the ecological phenomenon (Fuerst and Putnam, 1993). These authors reported that evidence must be put forward before any attempt is made to determine the causes of interference. The effects of allelopathy are manifested in the soil environment that provides a myriad of physical, chemical and biological processes that may interact with allelochemicals that could influence the study. It is impossible to prove that chemicals released by plants do not affect neigbouring plants. Harper (1997) proposed a rigorous protocol to search for the cause and effect. The cause – and – effect relationship cannot be established merely by observing appearance of phytotoxic symptoms on the one hand and showing the presence of chemicals of demonstrated toxicity in the vicinity of an affected plant on the other.

Once an inhibitory leachate or extract is obtained, a preliminary chromatographic separation can be made. Information about the polarity of the active allelochemical(s) can be obtained by extracting the leachate with a series of organic solvent ranging from non – popar to polar, for example, n – hexane, methylene chloride, methanol, and water. According to Hash (1985) and Shaw (1991), further separations will be dictated by polarity of the active fractions. As indicated by Shaw (1991), bioassays are used at each step of the purification process to determine which fraction contains allelochemicals and to monitor their activity. While there is no standard bioassay for allelochemical purification, the bioassay procedure used to determine the presence of allelochemical activity in the field may no longer be suitable for allelochemical purification, for example, wilting of whole susceptible plants watered with leachate from aggressor plant foliage requires relatively large quantities of leachate and the process of filtration to obtain the leachate would become cumbersome after several separations.

The effect of weeds on crops, crops on weeds and crop on crops have invariably been emphasized. Results obtained so far clearly demonstrate that some of the findings on allelopathic control of weeds, elimination of deleterious allelopathic effects of crops on crops or exploitation of beneficial interaction in a rotation or mixed cropping system have a direct bearing on crop production (Rizvi *et al.,* 2004). According to Aldric (1994), weeds interfere with crops by inhibiting germination and seedling establishment and also by the growth of the crop.

Cyperus esculentus (Yellow nutsedge) is a herbaceous perennial that is considered as one of the world's weeds. It is a problem in cropping systems in tropical climates, where it causes large losses in crop yields. The weed is characterized by prolific vegetative activity which produces a complex underground system of basal bulbs, rhizomes and tubers (Harper and Balke, 1981). Drost and Doll (2004) concluded that extract residues of *Cyperus esculentus* have an inhibitory effect on the growth of soyabeans (*Glycine max*) and maize. A similar research conducted by Kil and Yim (1983) show that toxic substances inhibited seed germination and growth of the species in the forest. These substances were released in fresh and fallen leaves, roots and pines forest soil. Kil (1989) studied the allelopathic potential of five species of pinaceaes, viz *P. densiflora, P thunbergii, P. rigida, Larix leptolepis* and *cedrus deodora*. All the five species inhibited germination of test species. It was also observed by Sale (2009) that application of leaf extract and leaf mulch under nursery condition has an inhibitory effects on the test crop.

Materials and Methods

Experimental sites

The nursery experiments were carried out at the Forestry Nursery and Arboretum site of the Federal University of Technology, Akure in the humid low land of Southwestern Nigeria. Akure is located on Latitude $7^{0}17^{1}$ N and Longitude $5^{0}10^{1}$ E in the rainforest belt at an elevation of 350 m above sea level. Akure lies in the tropical rainforest zone with mean annual temperation of about 31^{0}C (Min. 26.9^{0}C and Max. 34^{0}C). The relative humidity ranges between 68% and 86% during wet season and less than 50% during the harmattan period (Ogunnika, 2000). Mean annual rainfall is 1500mm with bimodal rainfall pattern (Oke, 2001 and Sale, 2009).

Method of Data Collection

The tree species used for the study (*Acacia auriculiformis* and *Gliricidia sepium*) leaves were collected from the Teaching and Research Farm of the Federal University of Technology, Akure and (*Eucalyptus citriodora*) leaves were collected from the arboretum of Forest Research Institute of Nigeria, Ibadan.

Procedure for Experiment

The experiment was conducted to evaluate the effect of different concentrations of leaf extracts and leaf mulch from dry leaves of *Acacia Auriculiformis, Eucalyptus citriodora* and *Gliricidia sepium* on seedling growth of millet in the potting medium. Millet (*Panicum miliaceum* L.) was planted in plastic pots filled with topsoil taken from the experimental site. Fifteen (15) seeds of millet was sown per pot and watered daily. After two weeks of growth, the plants were thinned to six (6) per pot by removing the weak plants. The plants were left without water for one day before extract of the various concentrations at different percentages (0, 6, 8, 10 and 12%) prepared by decaying 0, 15, 20, 25 and 30g) of crushed leaves respectively were applied at a single doze of 250 ml of extract per pot and also 250 ml of distilled water as control. The treatments were replicated three times and arranged in a randomized complete block design (RCBD). After two days of treatment application, all the plant were nurtured with water to field capacity daily for eight (8) weeks.

In a separate experiment, dried crushed leaves of the three test species were applied to the potted millet as mulch at the rate of 15, 20, 25 and 30 g per pot with a set of pot without mulch serving as control. The pots were replicated three times and arranged in a randomized complete block design (RCBD), and watered once daily for eight (8) weeks. At two weeks interval two uppermost leaves of plant in each treatment from the two (2) separate experiment were removed for determination of chlorophyll content. After six (6) weeks the plants were harvested and their dry matter was measured and also analyzed for N, P, K, alkaloid, polyphenol and carbohydrate contents.

Statistical Analysis

The data collect from the two experiments were subjected to analysis of variance procedure for randomized complete block design (RCBD) using SPSS statistical package.

Results

Comparison of the effects of different leaf extracts and mulch has showed that the percentage germination under *Acacia* and *Eucalyptus* extracts were significantly higher than those of corresponding *Gliricidia* extract treatment. Table 1 and 2 shows the chemical composition of leaf extract and mulch used for the experiment. All the chemical constituents were significantly higher ($P< 0.05$) in the *Gliricidia* extract than the corresponding *Acacia* and *Eucalyptus* extracts.

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Table 1: Chemical composition of leaf extracts of *Acacia, Eucalyptus* and *Gliricidia* used for the pot experiment

	Chemical constituents				
Species	**Polyphenol** (%)	**Alkaloid** (%)	**Nitrogen** (%)	**Phosphorus** (%)	**Potassium** (%)
Acacia	*0.79*[a]	*0.41*[a]	*0.06*[a]	*0.03*[a]	*0.04*[a]
Eucalyptus	*0.09b*	*0.51*[a]	*0.05*[a]	*0.03*[ba]	*0.03*[a]
Gliricidia	*0.10*[b]	*0.79*[b]	*0.07*[b]	*0.04*[b]	*0.06*[b]

Values are mean of three replicates; means with the same superscripts in column are not significantly different (P> 0.05)

Table 2: Chemical composition of leaf mulch of *Acacia, Eucalyptus* and *Gliricidia* used for the pot experiment

	Chemical constituents				
Species	**Polyphenol** (%)	**Alkaloid** (%)	**Nitrogen** (%)	**Phosphorus** (%)	**Potassium** (%)
Acacia	*5.45*[a]	*8.35*[a]	*3.90*[a]	*0.08*[a]	*0.61*[a]
Eucalyptus	*3.40*[b]	*7.41*[a]	*2.50*[b]	*0.07*[a]	*0.55*[a]
Gliricidia	*2.76*[c]	*5.61*[b]	*4.40*[a]	*0.10*[a]	*0.71*[a]

Values are mean of three replicates; means with the same superscripts in column are not significantly different (P> 0.05)

Effect of leaf extract and mulch on dry weight of millet seedlings in potting medium

At two weeks after planting (2 WAP), the dry weight of seedling in the control pots were significantly higher (P < 0.05) than in any of the treatment with *Acacia, Eucalyptus* and *Gliricidia* extracts. But increasing the concentration of the extract did not produce any significant effect on seedling dry weight. This was the trend observed in treatment with *Acacia* and *Eucalyptus* leaf extracts. But *Gliricidia* extract produced different significant effect in which the interference was increasing with extract concentration. As the extract concentration increased, there was a corresponding decline in seedling dry weight. After four weeks of planting (4 WAP), the pattern of dry weight accumulation was similar to what was obtained at 2 WAP in treatment with *Acacia* extracts. In the case of *Eucalyptus* and *Gliricidia* extract, there was a decline in seedling dry weight as concentration increased. At six weeks after planting, 0, 6, 8, 10 and 12% *Acacia* extracts did not significantly (P < 0.05) affect seedling of dry weight except at the highest extract concentration of 12%. For *Eucalyptus* and *Gliricidia,* only extract concentration of 10 and 12% reduce dry seedling weight relative to control.

There was no significant difference between control and mulched pots at two weeks after planting (2 WAP) irrespective of mulch rate. This was the trend observed in all the three species (*Acacia, Eucalyptus* and *Gliricidia*). However, the effect of mulch application emerged at 4 weeks after planting when the control treatment resulted in significantly higher seedling dry weight than in some of the mulch treatments (20, 25 and 30 g/pot for *Eucalyptus* and *Gliricidia* and 30 g/pot for *Acacia*) than the other mulch treatments. At 6 WAP, 25 and 30 g/pot of *Acacia* mulch application resulted in significant yield decline relative to the control. But in case of *Eucalyptus* and *Gliricidia* mulches, yield decline relative to control only occurred at 15 and 20 g/pot but application of higher rates of 25 and 30 g per pot significantly increased seedling dry weight over the control. Growth of millet at 6 weeks after planting in the pot experiment is as shown in plate 1.

Effects of leaf extract and mulch on the chlorophyll content of millet

The results of content are presented in table 3 and 4. None of the different concentrations of *Acacia, Eucalyptus* and *Gliricidia* extracts had significant effect on chlorophyll content of the potted millet seedlings two weeks after planting (2 WAP). At four weeks after planting, are similar results at 2 WAP, the various concentrations of *Acacia* had no significant effect (P > 0.05) on leaf chlorophyll content. However, between 6 – 12% extract of *Eucalyptus* and *Gliricidia* significantly (P < 0.05) reduced the chlorophyll content relative to control (Table 3). Generally, the trend was declining leaf chlorophyll content as extract concentration increased. This trend was maintained but less produced at six weeks

Plate 1: Growth of millet at 6 weeks after planting (WAP) in the pot experiment

Table 3: Total chlorophyll content (mg/100g tissue) of potted millet as influenced by leaf extracts of selected tree species

Period	Extract Concentration %	chlorophyll content (mg/100g tissue) *Acacia*	*Eucalyptus*	*Gliricidia*
2WAP	0	0.82^a	0.91^a	0.80^a
	6	0.87^a	0.92^a	0.73^a
	8	0.74^a	0.75^a	0.69^a
	10	0.77^a	0.78^a	0.80^a
	12	0.76^a	0.79^a	0.91^a
4WAP	0	1.31^a	1.30^a	1.36^a
	6	1.22^a	0.75^b	0.82^b
	8	1.21^a	0.76^b	0.89^b
	10	1.24^a	0.84^b	0.78^b
	12	1.43^a	0.99^b	1.01^b
6WAP	0	1.95^a	1.89^a	1.72^a
	6	1.60^a	1.90^a	1.58^a
	8	1.70^a	1.61^a	1.61^a
	10	1.76^a	1.58^a	0.82^b
	12	1.57^b	1.23^b	0.75^b

Values are mean of three replicates; means with the same superscripts in column are not significantly different (P > 0.05).

Table 4: Total chlorophyll content (mg/100g tissue) of potted millet as influenced by application of leaf mulch of selected tree species

Period	Mulch weight (g/pot)	chlorophyll content (mg/100g tissue) *Acacia*	*Eucalyptus*	*Gliricidia*
2 WAP	0	1.07^a	1.17^a	1.12^a
	15	0.84^a	1.10^a	0.95^a
	20	0.87^a	1.10^a	0.98^a
	25	0.95^a	0.99^a	0.96^b
	30	0.90^a	1.01^a	0.75^b
4 WAP	0	1.29^a	1.24^a	1.18^a
	15	1.32^a	1.45^a	1.29^a
	20	1.23^a	1.13^a	0.87^b
	25	0.97^{ab}	0.85^b	0.83^b
	30	0.76^b	0.83^b	0.88^b
6 WAP	0	2.03^a	1.90^a	1.84^a
	15	1.88^a	2.01^a	1.64^a
	20	1.75^a	2.12^a	1.76^a
	25	1.85^a	1.80^a	1.69^a
	30	2.17^a	1.92^a	1.81^a

Values are mean of three replicates; means with the same superscripts in column are not significantly different (P > 0.05).

after planting. At this stage the highest extract concentration resulted in highest significant decline in leaf chlorophyll content. In order of severity of effect, it was *Gliricidia* extract > *Eucalyptus* > *Acacia*.

The leaf chlorophyll content and how it was affected by different rates of mulch application is shown in table 4. At two weeks after planting, only *Gliricidia* at 25 and 30 g per pot significantly decreased the leaf chlorophyll relative to the 0 g mulch per pot (control). At 4 WAP, the trend changed from what it was at two weeks. At this stage, application of 30g per pot of *Acacia* mulch; 25 and 30 g of *Eucalyptus* and 20, 25 and 30 g of *Gliricidia* mulch significantly reduced chlorophyll content compared to the control. Six weeks after planting, there was no significant effect following the application of different rates of leaf mulch.

Discussion

The main focus in the pot experiment was to evaluate the inhibitory potential of leaf extract and mulch of *Acacia, Eucalyptus* and *Gliricidia* with millet as a test crop. Two weeks after application, leaf extract depressed the dry weight of millet seedlings relative to the control. The observed phytotoxic interference did not increase with extract concentration except for *Eucalyptus* and *Gliricidia* extracts. The results also show that in terms of duration, the inhibitory effect on dry weight waned with time and was more pronounced at two weeks after planting (2 WAP) than at six weeks. It is also evident from the results that the severity and persistence of the inhibitory effect of the extract was in order: *Gliricidia* > *Eucalyptus* > *Acacia.*

The phytotoxic interference of mulch application differs in some respects from that of extract. For example, two weeks after application, none of the mulch types produced any inhibitory effect on millet seedling weight until at 4 weeks after planting (4 WAP). It is evident from these results that there are differences between leaf extract and mulch in their inhibitory effects. These differentials can be attributed to differences in the litter quality of the mulches. In an earlier work, Oyun (2001) had rated the quality of these mulches base on their chemical composition as *Gliricidia* > *Eucalyptus* > *Acacia.* On basis of quality criterion, *Gliricidia* mulch is expected to decompose and release nutrients faster than *Eucalyptus* and *Acacia*.

The leaf chlorophyll content of millet, as observed in this study, was reduced by application of both leaf extract and mulch of *Acacia, Eucalyptus* and *Gliricidia.* However, the inhibitory effects of these on chlorophyll content were not as spectacular as the way in which they interfere to reduce dry matter yield in pot experiment. For example, increasing the concentration of extract or amount of mulch did not always result in significant decline in chlorophyll content. When the mean effects on chlorophyll content following the application of leaf extract and mulch of the three species were compared, reduction in chlorophyll content relative to the control was highest in pots that received either *Gliricidia* mulch or extract. This was followed in order by *Eucalyptus* and *Acacia,* thus confirming the relative phytotoxicity of the leaf extract and mulch of these species.

Conclusion

The total chlorophyll content of millet plant reduced due to the application of leaf extract and leaf mulch of *Acacia, Eucalyptus* and *Gliricidia.* The inhibitory effect has been attributed to the presence of alkaloids and polyphenols which are considered to be allelochemicals. Foliar content of both alkaloids and polyphenols in millet plant were increased following application, particularly, of *Gliricidia* mulch.

Although, it is generally acknowledge that tree legumes (*Acacia, Gliricidia, Leucaena*) can improve the nutrient status of the soil particularly through litter return, however it has been queried that allelochemicals which are associated with decomposition process are being released alongside with nutrients and these allelochemical are suspected to have negative effect on physiological process of crop even while the nitrogen so released improve the soil status.

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