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Inhibitory Effects of Marama (*Tylosema esculentum*) on the Growth of Oat (*Avena sativa*) and Barley (*Hordeum vulgare*)

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Abstract: This study was conducted in order to evaluate the allelopathic potential of marama bean (*Tylosema esculentum* (Burch) A. Schreib), a drought-avoider legume of Africa, which produces seed and tubers with potential for use as human food and animal fodder. Fresh, senesced and decaying leaves from marama were evaluated for their allelopathic potential on oat (*Avena sativa*) and barley (*Hordeum vulgare*) through *in vivo* studies. The studies were performed in growth chambers, using 10, 20 and 30 g of leaf tissue mixed with screened perlite as a substrate. The effects of the different leaf types were evaluated by determining root fresh and dry weight, shoot length and shoot fresh and dry weight. The results indicated the allelopathic potential of the marama leaves, being more pronounced in the fresh, moderate in the senesced and lower in the decaying leaves.

Key words: Marama, allelopathic potential, leaves, oat, barley

INTRODUCTION

Marama bean (*Tylosema esculentum* (Burch.) A. Schreib) is a wild perennial legume, indigenous to the arid and semi-arid grasslands of southern Africa. The remarkable socio-economic value of the plant in African communities is mainly explained by the fact that the bean is highly nutritious when cooked, with protein and oil content comparable to soybean (*Glycine max*) and groundnut (*Arachis hypogaea*), respectively (Powell, 2008; Ketshajwang *et al.*, 1998; Travlos *et al.*, 2007a, b). Because of its great ability to survive under unfavorable conditions it can be considered suitable for cultivation, especially under preventive conditions for other crops (Mitchell *et al.*, 2005; Travlos *et al.*, 2007a, b).

Plants produce many compounds that have no obvious metabolic, physiological or structural role for the producer. These secondary metabolites might exhibit allelopathic effects and influence other organisms, rendering allelopathy one of the predominant forces in the development of plant communities (Shiraishi *et al.*, 2002; Travlos and Paspatis, 2008). No studies to date have assessed the allelopathic potential of *T. esculentum*, while studies on related species, likewise *Bauhinia purpurea* and *B. variegata* have already conducted (Kaletha *et al.*, 1996), showing that several allelochemicals

were released and they can probably be used and have ecological (e.g., herbicidal) importance.

This study reports a preliminary investigation into the allelopathic features of *T. esculentum*. The aim of the present study was to evaluate the allelopathic activity of marama tissues on the growth and development of *Avena sativa* L. and *Hordeum vulgare* L.

MATERIALS AND METHODS

In order to evaluate the allelopathic potential effects of *T. esculentum* leaves, a preliminary study was conducted. This study was performed in growth chambers with the use of small plastic pots (70 mm ID and 110 mm high), filled with sieved perlite (Isocon S.A., Athens, Greece) having a uniform particle distribution (0.5 to 1.0 mm). Leaves were collected from *T. esculentum* plants grown in the experimental field of Agricultural University of Athens in 2004.

For the purposes of allelopathic assessment, three different physiological stages of the marama leaves were used: fresh marama leaves were collected from the plants, senesced leaves, which had a yellowish colour, were collected from the soil surface around the plants and decaying leaves having a dark grey-brown colour were collected from the experimental field, too.

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The leaves were cut into 5x5 mm segments and placed at -18°C for 7 days. The frozen tissues were incorporated in the perlite substrate at 3 different rates namely 10, 20 and 30 g of fresh weight per pot. The pots were then seeded with oat (*A. sativa*) and barley (*H. vulgare*) at a rate of 5 seeds per pot, respectively.

The seeded pots were placed in the growth chamber with a constant temperature of 25°C. All pots were irrigated with deionised water and artificial light was supplied with a photoperiod of 16 to 8 h light and dark, respectively. The seedlings were fertilized with foliar fertilizer (Nutrileaf 20-20-20; Miller, Hanover, Pa.) at a rate of 2.5 g L⁻¹ at weekly intervals after the emergence of the second leaf. The seedlings were destructively sampled 12 days after seeding oat and barley. The growth parameters that were measured included: root fresh weight; root dry weight; shoot length; shoot fresh weight and shoot dry weight. Each treatment had four replicates arranged in a completely randomized design.

Data from both experiments were analysed together and treatment values for all features were expressed as means between the 2 years. Statistical analysis of the results was performed using one-way ANOVA, while mean comparison was performed using Fisher's least signification different (LSD) test at p<0.05 by means of Statistica 6.0® software package (Statsoft., Tulsa, OK, USA).

RESULTS AND DISCUSSION

Present results indicated that the incorporation of marama leaves into the perlite substrate reduced growth and development of oat and barley, thus suggesting that toxic compounds were released from the marama tissue. Oat and barley were included in this study since they have been used extensively in allelopathy research as the receiver plants to test compounds released by a donor plant. Moreover, oat and barley seeds germinate relatively evenly, resulting in a uniform and rapid plant growth that enables qualification of biological response in plants. In addition, oat biotest is considered as a sensitive and easily facilitated method (Travlos *et al.*, 2007a; Travlos and Paspatis, 2008).

Growth inhibition was dependent on the type of leaves and it was proportional to the incorporation rate. More specifically, the greatest reduction in root and shoot growth was observed in both cereals from the fresh marama leaves (Table 1, 2). The senesced leaves resulted in moderate and the decaying in low growth inhibition. This finding of the highest levels of allelochemicals in young leaves and the progressively declined phytotoxicity with leaf age is in accordance with other recent studies on several species (Barney *et al.*, 2005; Cipollini and Gruner, 2007). Within each type of marama leaves, the shoot and the root growth inhibition

Table 1: *Avena sativa* root and shoot characteristics as affected by marama leaf type (control, decaying, senesced, fresh) and the concentration of the incorporated tissue

Marama leaf type	Marama leaf tissue (g pot ⁻¹)	Root fresh weight (mg)	Root dry weight (mg)	Shoot length (mm)	Shoot fresh weight (mg)	Shoot dry weight (mg)
Control	0	39.23a	6.93a	286a	66.72a	15.66a
Decaying	10	33.17b	6.18ab	232b	61.25b	14.21a
	20	30.08b	5.12bc	229b	57.44c	12.00b
	30	28.11bc	4.58cd	177c	52.21d	10.35bc
Senesced	10	31.06b	5.91ab	226b	55.16c	12.43b
	20	26.71c	4.78cd	212bc	51.03d	10.02bc
	30	25.64c	3.53e	174c	48.71e	8.89cd
Fresh	10	27.89bc	4.88cd	205bc	52.65d	11.90b
	20	25.90c	3.95de	189bc	50.21de	9.37c
	30	23.86cd	3.38e	153d	46.60e	7.94cd

Values are the means of four replications and means that differ significantly using Fisher's LSD test at a probability level p<0.05 are noted with different letter(s)

Table 2: *Hordeum vulgare* root and shoot characteristics as affected by marama leaf type (control, decaying, senesced, fresh) and the concentration of the incorporated tissue

Marama leaf type	Marama leaf tissue (g pot ⁻¹)	Root fresh weight (mg)	Root dry weight (mg)	Shoot length (mm)	Shoot fresh weight (mg)	Shoot dry weight (mg)
Control	0	44.11a	7.30a	304a	71.31a	20.26a
Decaying	10	42.46a	6.38ab	266b	70.66a	18.21a
	20	39.86b	5.91bc	255b	64.23b	16.00b
	30	37.23bc	4.88cd	221bc	60.11c	14.13b
Senesced	10	39.41b	5.98bc	231b	60.08c	15.93b
	20	35.81c	4.82cd	191cd	59.21c	12.82bc
	30	33.91c	3.99d	180cd	54.33d	11.81c
Fresh	10	39.07b	4.80cd	209c	58.12c	14.89b
	20	34.89c	4.15d	186cd	55.09d	13.06bc
	30	30.12d	3.94d	169d	51.85e	11.38c

Values are the means of four replications and means that differ significantly using Fisher's LSD test at a probability level p<0.05 are noted with different letter(s)

increased proportionally with the leaf mass that was incorporated in the substrate. Therefore, the greatest reduction was observed at the high incorporation rate (30 g pot⁻¹) of the fresh marama leaves for all the growth parameters monitored.

However, some differences between the two species (oat and barley) were observed. It was noticed that in oat the growth parameters that were mostly affected by the high incorporation rate of the leaves were the root dry weight, the shoot length and the shoot dry weight (51, 47 and 49% growth reduction compared to the control, respectively). In barley, the corresponding values were 46, 44 and 44 %, respectively. Oat was verified as a more sensitive bioindicator than barley, since its root and shoot growth were greatly inhibited (Table 1). Indeed, oat root growth inhibition was more intense compared to shoot growth, which is in accordance with previous findings (Travlos *et al.*, 2007a; Travlos and Paspatis, 2008). These different responses between the two species exhibited the species-specific inhibitory action of marama phytotoxic substances. These results are in accordance with the results of similar studies (Chung and Miller, 1995; Travlos *et al.*, 2007a; Travlos and Paspatis, 2008; Travlos *et al.*, 2008), who demonstrated that the growth regulatory effects of allelochemicals possess species-specific action.

From this study, it was demonstrated that inhibitory compounds existed within the marama leaves and that these compounds were more phytotoxic in the fresh rather than the senesced or decaying leaves. These observations are in agreement with others at several plant species, supporting that the toxic effect of plant residues generally decreased as decomposition progressed (Mersie and Singh, 1987; Nektarios *et al.*, 2005; Cipollini and Gruner, 2007). The reduction of the inhibitory action according to the progress of decomposition may be due either to the release of toxic substances from the decomposing tissues and/or to the decrease of the decomposition progress itself (Mersie and Singh, 1987; Nektarios *et al.*, 2005). After the present results, marama could be included to the group of tuberous plants which are involved in interactions with other plant species through the production and release of allelochemicals (Reinhardt and Bezuidenhout, 2001; Norsworthy and Median IV, 2005; Dias and Dias, 2007; Javaid *et al.*, 2007). Under the view of integrated weed management, allelopathy could play a pivotal role. Therefore, the study of the allelopathic effects of underutilized species, such as *Tylosema esculentum* on several plant species seems quite interesting, while it could have further, potential value if it is accomplished with future specific studies.

CONCLUSION

The study clearly indicated that water soluble inhibitory substances were present within the leaves of *T. esculentum*. Further research is needed, in order to determine the toxicity potential and characterize the aqueous extracts of several tissues of marama and to categorize other species in terms of their response to marama inhibitory compounds.

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