

ALLELOPATHIC POTENTIAL OF *Brachiaria brizantha* AND *B. milliformis* ON SEED GERMINATION OF SELECTED BIOASSAY SPECIES

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ABSTRACT

Brachiaria brizantha and *B. milliformis* are widely grown improved pasture species of the coconut plantations in Sri Lanka. Field observations indicate that these two grass species suppress ground vegetation in coconut plantations. Therefore, the aim of this study was to test the allelopathic effect of *B. brizantha* and *B. milliformis* using their root components from where they possibly release allelochemicals to the environment. Soils where *B. brizantha* and *B. milliformis* are grown and root exudates, aqueous extracts of fresh and dry roots were investigated to verify their allelopathic effect on seed germination of five bioassay species; *Raphanus sativus*, *Capsicum annum*, *Lycopersicom esculantum*, *Crotalaria junica* and *Chromoleana odorata*. Fifty seeds from each bioassay species were placed in a petri dish containing root exudates, root extracts and contaminated soil and the percentage of seed germination was examined after 3 days. The experiment was repeated four times. Seed germination percentage of *Capsicum annum*, *Lycopersicom esculantum* and *Chromoleana odorata* was significantly inhibited by *B. brizantha* contaminated soil. However, the degree of inhibition varied among the bioassay species. The fresh aqueous root extracts of *B. brizantha* was highly phytotoxic and it significantly reduced seed germination of all the bioassay species than the dry root extract. The maximum reduction in seed germination of all the bioassay species was caused by root exudates of the two grass species. *B. brizantha* and *B. milliformis* species incorporated root aqueous extracts; root exudates and its rhizosphere soil suppress seed germination of the five bioassay species and suggest that these responses are attributed to allelopathic effects which should be investigated further in the field.

Key words: *Brachiaria brizantha*, *B. milliformis*, allelopathy, seed germination, root extracts.

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INTRODUCTION

Coconut is a tropical perennial plantation crop and its canopy structure requires wide spacing between palms, which permit abundant sunlight to the understory. It does not fully utilize all incoming radiation or all of the available moisture and nutrients. As a result, the unutilized space beneath the plantation is invaded by a wide range of perennial and annual weed species (Senarathne *et al.*, 2003). The establishment and maintenance of a good herbaceous pasture in the coconut under story can provide livestock feed, while also preventing the invasion of non-nutritious, yet aggressively competitive weeds (Plucknet, 1974). The positive effect of integration is therefore the "replacement" of non productive weed species with grass species such as *B. brizantha* and *B. milliformis*. Both are high biomass productive perennial, vigorously grown grasses found in coconut plantations in Sri Lanka. The introduction of high yielding grasses into mature plantations may be expected to exert a stronger competitive effect than natural vegetation, primarily due to the increased demand for nutrients and moisture. *B. brizantha* grown in monoculture with routine agronomic practices caused a 13% nut yield reduction in mature coconut plantations, which could be due to significant absorption of soil water (Vidhana, 1998). Humphreys (1991) stressed that the yield of plantation crops may be positively or negatively affected by pasture systems, depending on the nature of the interference and the net effects on the crop environment. Therefore, managing pasture under coconut is very important to achieve maximum herbage production of good quality, without affecting the coconut yield. It is to be noted that aggressive pasture species such as *B. brizantha* are likely to compete with coconut, unless they are well managed (Liyanage, 1999). If allelopathic effects persist, the adverse effect could be accelerated and provide unprofitable results for the coconut growers.

The term of "allelopathy" coined by Molish (1937) generally refers to any direct or indirect effect of the plant (including microorganisms) on the germination, growth or development of other plants, through the production of chemicals that escape into the environment (Rice, 1984). Allelochemicals can be released either through leaching, decomposition of plant residues, volatilization, or root exudation (Chou, 1999). There is increasing attention to explain the development of plant communities and as an important aspect is a weed-crop interaction (Aldrich, 1987; Rice, 1987). However, there has been no basic information about the phytotoxic activity of *B. brizantha* and *B. milliformis* in coconut soils. A better understanding of the allelopathic potential of *B. brizantha* will provide a basis for improving knowledge of plant population changes in coconut plantations. The

present study was conducted to determine if aqueous extract of fresh and dry roots of *B. brizantha* and *B. milliformis* and contaminated soil and root exudates were allelopathic to the growth of bioassay species.

MATERIALS AND METHODS

The experiments were carried out in the plant house and laboratory of the Coconut Research Institute located in the Low country Intermediate Zone of the North Western province of Sri Lanka from March to August, 2009. In the plant house, petri dishes received photosynthetically active radiation (PAR) ranging from 500-1150 $\mu\text{molm}^{-2}\text{s}^{-1}$ and the average day and night temperatures were in the range of 30-34°C and 26-30°C respectively. Relative humidity varied between 35-60% during the day and 20-27% during the night. In the bioassay, *Raphanus sativus*, *Capsicum annum*, *Lycopersicom esculentum*, *Crotalaria junica* and *Chromoleana odorata* seeds were used as the test species due to their high sensitivity to the phytotoxic activity of *B. brizantha* and *B. milliformis*, as observed in preliminary study. Seeds of the selected weed species namely *Chromoleana odorata* and *Crotalaria junica* were collected from five different locations in the major coconut growing regions of Sri Lanka between February to March 2009 and were stored at 5°C under dark conditions. Seeds of Radish (*Raphanus sativus*), Chillies (*Capsicum annum*), and Tomato (*Lycopersicom esculentum*) were taken from the Seed and Plant Material Development Centre, Department of Agriculture, Sri Lanka. The selected treatments of the experiments were arranged in a Completely Randomized Design (CRD) with ten replicates (each Petri dish and pot representing one replication of a single species in each trial) in the respective studies.

Effect of residual toxicity of contaminated soil on seed germination of bioassay species

B. brizantha and *B. milliformis* grown soil was classified as Madampe soil series (light textured high productive soil series; bulk density = $1.48 \pm 0.02 \text{ g/cm}^3$; total available water = $5.71 \pm 0.89\%$; penetrometer resistance = $240 \pm 16.3 \text{ N/cm}^2$) was located in Bandirippuwa Estate, Lunuwila in the low country Intermediate climate zone (08°02N, 79°E, 35m altitude) (Vidhana, 2009). Contaminated soil was collected to a depth of 10cm from a field where *Brachiaria spp.* had been grown for the last five years and soil from a field that did not have *Brachiaria spp.* was used as a control. Soil was dried at room temperature and sieved through a 2mm mesh. Ten grams each of test and control soils were uniformly spread in 9cm diameter petri dishes, separately. Fifty seeds of selected bioassay species were placed uniformly on the soil. Seeds were covered with the same soil. Soil

was adequately moistened with distilled water. The dishes were kept in plant house at 27-30°C. Each treatment was replicated ten times.

Effect of aqueous extracts of dry roots on seed germination of bioassay species

Root portions of the selected grass species were cut into small pieces with scissors, dried under full sunlight for 1 week, ground to a powder with an electrical grinder (Thomas Wiley, Thomas Company, U.S.A). The dried powdered roots were immersed in distilled water in the ratio of 1:20 w /v and agitated for 24 hours on an orbital shaker at room temperature (29°C). The extract was strained through two layers of filter paper (Whatman No. 02). The extract was refrigerated at 5°C until use. One concentration of the dry root aqueous extract was used in this experiment. Fifty seeds each of selected bioassay species were placed separately in 9cm diameter petri dishes lined with cotton wool. Treatments were applied in 5 ml volumes per dish and distilled water was used for the control. The petri dishes were kept in a plant house for 72 hours at 28-30°C. Treatments were replicated ten times.

Effect of aqueous extracts of fresh roots on seed germination of bioassay species

The fresh root parts of *B. brizantha* and *B. milliformis* cut into 1-2cm lengths were put into distilled water in the ratio of 1:2 w /v soaked in a flask and agitated for 24 hours on an orbital shaker at room temperature (29°C). The extract was strained through two layers of filter paper (Whatman No. 02). The extract was refrigerated at 5°C until use. One concentration of the fresh root aqueous extract was used in this experiment. Fifty seeds each of selected bioassay species were placed separately in 9cm diameter petri dishes lined with cotton wool. Treatments were applied in 5 ml volumes per dish and distilled water was used for the control. The petri dishes were kept in a plant house for 72 hours at 28-30°C. Treatments were replicated ten times.

Effect of root exudates on seed germination of bioassay species

Plants of *B. brizantha* and *B. milliformis* were planted in the plastic pots kept in the plant house. After 2 months well grown mature 5 plants were selected from the 2 species. Plants were placed in Aluminum potting racks, the bottom of the pots were covered by using polythene bags. Thereafter, 100ml of distilled water was added to the pots to bring the soil to field capacity and the root exudate was obtained. The exudate was refrigerated at 5°C until use. One concentration of the root exudates was used in this experiment. Fifty seeds each of selected bioassay species were placed separately in 9cm diameter petri dishes lined with cotton wool. Treatments were applied in 5ml volumes per dish and distilled water was used for the control. The petri dishes were kept in a plant house for 72 hours at 28-30°C. Treatments were replicated ten times.

Data collection

The Petri dishes were kept in a plant house and supplied with adequate light for seed germination. Germination of *Raphanus sativus*, *Capsicum annum*, *Lycopersicom esculentum*, *Crotalaria junica* and *Chromoleana odorata* were recorded daily; during 12 days according to the method of the Association of Official Seed Analysis (1985).

Numbers of germinated seeds were converted to % as per following formula.

$$\text{Germination \%} = \frac{\text{No of Germinated seeds}}{\text{Total No. of seeds}} \times 100$$

Statistical analysis

Data analysis of the above experiment was conducted using Analysis of Variance (ANOVA) using Statistical software and the significance was tested using the Least Significant Differences (LSD) at $P=0.05$ (SAS Institute, 1999).

RESULTS AND DISCUSSION

Residual toxicity of contaminated soil on seed germination

Soil collected from the *B. brizantha* rhizosphere had a strong inhibitory effect on the seed germination of some bioassay species such as *Lycopersicom esculentum*, *Capsicum annum* and *Chromoleana odorata* (Table-1). However, there was no significant difference of the allelopathic effect of *B. brizantha* and *B. milliformis* on *Crotalaria junica* and *Raphanus sativus* seeds. The lowest germination percentage (17%) was recorded in *Chromoleana odorata* seeds, when those seeds were sown on the *B. brizantha* contaminated soil, while the highest germination percentage was found in *Lycopersicom esculentum* seeds, when compared with that of *Capsicum annum* seeds (44%) (Table-1). Furthermore, with *B. milliformis* contaminated soils, the lowest inhibition of germination percentage (23%) was found in *Chromoleana odorata* seeds and the highest germination percentage (73%) was recorded in *Lycopersicom esculentum* seeds (Table-1). This is in agreement with the results of Chung and Miller (1995) who reported the inhibitory effect of soil collected from the surrounding area of alfalfa plants on their test bioassay species. This inhibition may be due to the release of phytotoxic substances by the root itself or through interaction between microorganisms and tissue litter.

However, this interpretation needs further study because several factors are involved in allelopathic activity and seed germination. In addition, the alteration of the physico-chemical characteristics of the soil may affect the quantitative and qualitative status of phyto-chemicals, which, in turn influences the allelopathic

expression of plants (De-Moral and Muller, 1970). However *B. brizantha* inhibited germination of the above species to a greater extent than of *B. milliformis*. Overall results showed that the contaminated soil of *B. brizantha* and *B. milliformis* adversely affected the seed germination of *L. esculentum*, *C. annuum*, and *C. odorata*. Therefore, it can be concluded that allelopathic nature of soil was due to the leaching of toxins from *B. brizantha* and *B. milliformis*.

Table-1. Effect of residual toxicity of contaminated soil on seed germination of selected species.

Treatments	Seed germination %				
	<i>Raphanus sativus</i>	<i>Lycopersicom esculentum</i>	<i>Capsicum annum</i>	<i>Crotalaria junica</i>	<i>Chromoleana odorata</i>
T ₁ Control	66 a	81a	66a	21 a	62 a
T ₂ <i>B. brizantha</i>	40 b	44b	23 b	17 a	17 b
T ₃ <i>B. milliformis</i>	64 ab	73a	24 b	19 a	23 c
Significance	ns	**	**	ns	**
LSD (P = 0.05)	-	16.59	23.44	-	17.14

*Significant ** Highly Significant.

Within a column, means followed by the same letter are not significantly different by LSD (P=0.05).

Effect of dried root extract on seed germination of selected bioassay species

Air dried root extract of *B. brizantha* and *B. milliformis* significantly ($p \leq 0.05$) reduced the seed germination of bioassay species when compared to the control (only distilled water) (Table-2). Application of *B. brizantha* dried root extract significantly reduced the seed germination and the lowest germination percentage (7%) was found in *C. junica*, while the highest germination percentage (72%) was found in *R. sativus* seeds (Table-2). With the air dried root extract of *B. milliformis*, the lowest germination percentage (19%) was also observed in *C. junica* seeds, while the highest germination percentage (82%) was recorded in *R. sativus* seeds (Table-2).

Moreover, *B. brizantha* significantly suppressed seed germination of *C. junica* and *C. odorata* when compared to that of *B. milliformis*. These results are supported by the findings of Helgeson and Konzak (1950) who reported that aqueous extracts of field bindweed (*Convolvulus arvensis*) and canada thistle (*Cirsium arvense*) inhibited the germination of seeds and growth of seedlings of many crops. Overall results suggested that allelopathic effect of dried roots extract of *B. brizantha* and *B. milliformis* significantly ($p \leq 0.05$) suppressed the seed germination of *C. junica*, *C. odorata*, *L. esculentum*, *C. annuum* and *R. sativus* (Table-2).

Table-2. Effect of dried roots extract on seed germination of bioassay species.

Treatments	Seed germination %				
	<i>Raphanus sativus</i>	<i>Lycopersicom esculentum</i>	<i>Capsicum annum</i>	<i>Crotalaria junica</i>	<i>Chromoleana odorata</i>
T ₁ Control	95a	82a	77a	45a	46b
T ₂ <i>B. brizantha</i>	72b	27b	36b	7c	23b
T ₃ <i>B. milliformis</i>	82b	42b	44b	19b	33ab
Significance	**	**	**	**	*
LSD (P = 0.05)	11.82	20.07	10.25	11.30	14.63

*Significant ** Highly Significant

Within a column, means followed by the same letter are not significantly different by LSD (P=0.05).

Effect of fresh root extract on seed germination of selected bioassay species

Fresh root extracts of *B. brizantha* and *B. milliformis* significantly ($p \leq 0.05$) reduced the germination percentage of seeds of selected species when compared to the control (only distilled water). However, there was no significant effect of treatments on seed germination of *C. junica*. The lowest germination percentage (18%) was recorded in *C. odorata* seeds, with *B. brizantha* fresh root extract. The highest germination percentage was found in *L. esculentum* seeds when compared to that of *R. sativus* and *Capsicum annum* seeds (37%). Applications of fresh root extract of *B. milliformis* on to the seeds of the above species reveal that the lowest inhibition of germination percentage (30%) was found in *C. odorata* seeds whilst the highest germination percentage (58%) was recorded in *C. annum* seeds (Table-3).

Table-3. Effect of fresh root extract on seed germination of bioassay species.

Treatments	Seed germination %				
	<i>Raphanus sativus</i>	<i>Lycopersicom esculentum</i>	<i>Capsicum annum</i>	<i>Crotalaria junica</i>	<i>Chromoleana odorata</i>
T ₁ Control	68a	80a	82a	17a	44a
T ₂ <i>B. brizantha</i>	35b	37c	26c	4b	18c
T ₃ <i>B. milliformis</i>	42b	53b	58b	12ab	30b
Significance	**	**	**	ns	**
LSD (P = 0.05)	13.30	13.20	16.47	-	11.98

*Significant ** Highly Significant

Within a column, means followed by the same letter are not significantly different by LSD (P=0.05)

There was a significant difference ($p \leq 0.05$) in the germination of *L. esculentum*, *C. annum* and *C. odorata* seeds in *B. brizantha* and *B. milliformis* treatments. These findings are supported by the findings of Noor and Khan (1994) who reported a high reduction in *Zea mays* seed germination by *A. samana* fresh root extracts. The results presented in Table-3, indicate that the allelopathic effect of fresh roots

extract of *B. brizantha* and *B. milliformis* were adversely affecting the germination of *C. odorata*, *Capsicum annum*, *R. sativus* and *L. esculentum* seeds.

Effect of root exudates on seed germination of selected bioassay species

Root exudates of *B. brizantha* and *B. milliformis* reduced the germination percentage of seeds when compared to control treatment (distilled water). With the application of *B. brizantha* root exudates to the seeds, the lowest germination percentage (2%) was found in *C. junica* whilst the highest germination percentage was in *L. esculentum* seeds when compared with *R. sativus* and *C. odorata* seeds which were 60% (Table-4). After the application of *B. milliformis* root exudates to the seeds, the lowest germination percentage (5%) was found in *Crotalaria junica* seeds whilst the highest germination percentage (71%) was recorded in *Lycopersicom esculentum* seeds.

Table-4. Effect of root exudates on seed germination of bioassay species.

Treatment	Seed germination %				
	<i>Raphanus sativus</i>	<i>Lycopersicom esculentum</i>	<i>Capsicum annum</i>	<i>Crotalaria junica</i>	<i>Chromoleana odorata</i>
T ₁ Control	79a	89a	79a	21a	58a
T ₂ <i>B. brizantha</i>	44b	60b	42c	2b	29b
T ₃ <i>B. milliformis</i>	53b	71b	55b	5b	37b
Significance	*	**	**	**	**
LSD (P = 0.05)	20.27	13.1	9.98	9.15	15.6

*Significant ** Highly Significant

Within a column, means followed by the same letter are not significantly different

B. brizantha and *B. milliformis* root exudates caused a significant difference ($p \leq 0.05$) in the germination of *C. annum* seeds. However, the highest germination percentages were recorded in the control treatment, which were *R. sativus* (79%), *L. esculentum* (89%), *C. annum* (79%), *C. junica* (21%) and *C. odorata* (58%), respectively. These results agree with those of Helgeson and Konzak (1950), who found that root exudates of Canada thistle (*Cirsium arvense*) injured oat plants in the field while root exudates of *Euphorbia* and *Scabosia*. Our results suggested that allelopathic effect of roots exudates of *B. brizantha* and *B. milliformis* adversely affected the germination of all the bioassay species seeds.

CONCLUSIONS

The selected bioassay species were more sensitive to inhibitory effects of root extracts, exudates and contaminated rhizosphere soil of *B. brizantha* than those of *B. milliformis*. Hence, *B.*

bizantha has a greater allelopathic potential and releases allelopathic substances to the environment. However, the sensitivity to allelochemicals and extent of inhibition varied between species. The allelopathic effect of *B. brizantha* may be an important mechanism involved in invasive success of this plant. Under natural conditions, where a greater number of interactions with other organisms occur, these allelopathic effects can enhance or restrain plant growth and species diversity. Field experiments must be carried out to test the effectiveness of the allelopathic potential of above grass species under natural conditions.

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