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## Allelopathic Activity of Sunflower (*Helianthus annuus* L.) on Growth and Nodulation of Bambara Groundnut (*Vigna subterranea* (L.) Verdc.)

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**Abstract:** Laboratory and greenhouse experiments were conducted to determine the allelopathic activity of sunflower (*Helianthus annuus*) on seed germination, seedling growth, flowering and nodulation of bambara groundnut (*Vigna subterranea* L. Verdc.). Bambara groundnut seeds were treated with diluted and undiluted sunflower extracts under laboratory conditions. Sunflower was also mixed-planted with bambara groundnut in the greenhouse. The effect of sunflower plant residues on bambara groundnut was also determined. Sunflower leaf and root extracts reduced seed germination, seedling growth and reduced seedling dry weight of bambara groundnut under laboratory conditions. Root length was reduced more than the shoot length. Diluted extracts also inhibited seed germination and seedling growth in a concentration-dependent manner. There was no significant difference in the shoot length, number of leaves, nodules and flowers of bambara groundnut which was mix-planted with sunflower compared to bambara groundnuts grown on their own under greenhouse conditions; except for dry weight which was reduced. However, there was an increase in dry weight of sunflower that was mix-planted with bambara groundnut compared to sunflower grown on their own. Flowering and nodulation was completely inhibited in bambara groundnuts grown in soils mixed with sunflower plant residues at 75:25 and 50:50 (w/w) soil:residue mixture. Seed germination, shoot elongation and leaf formation was also reduced. Similar results were also obtained when the amended soil was re-used to grow bambara groundnut. The results of this study suggest that sunflower contains allelochemicals that have strong inhibitory effect on growth, especially flowering and nodulation, of bambara groundnuts. The results indicate the implications of cropping systems between sunflower and bambara groundnuts.

**Key words:** Sunflower, bambara groundnuts, *Helianthus annuus*, *Vigna subterranea*, allelopathy

### INTRODUCTION

Allelopathy is defined as production of secondary metabolites by plants and microorganisms that influence growth and development of biological systems (Torres *et al.*, 1996). Plants may have inhibitory effects on neighbouring plants by releasing allelopathic substances into the soil either as exudates from the living tissues or as decomposing plant residues (Rice, 1984; Inderjit, 1996). The allelochemicals are leached by rain into the soil where they may affect seed germination and growth of other plants (Inderjit and Keating, 1999; Inderjit and Duke, 2003). The effects may be due to a variety of processes that may include reduced cell division in the roots, suppressed hormonal activity, reduced ion uptake, inhibition of photosynthesis and respiration, inhibition of protein synthesis, inhibition of enzyme activity and reduced cell membrane permeability. The most frequently

reported allelochemicals are flavonoids, phenolic compounds and terpenoids (Anjum *et al.*, 2005; Djurdjevic *et al.*, 2004; Li *et al.*, 1992; Rice, 1984; Scrivanti *et al.*, 2003). Flavonoids have frequently been implicated in inhibiting seed germination and root growth (Rice, 1984). Phenolic compounds have been shown to inhibit germination and growth of a many plants (Reigosa *et al.*, 1999; Weir *et al.*, 2003), germination of *Pinus larico* (Muscolo *et al.*, 2001) and soil nitrification (Mao *et al.*, 2006) The detrimental interference of quack grass *Agropyron repens* on growth and nodulation in legumes has also been reported in both field and controlled environments (Weston and Putnam, 1985). Exogenously applied ethylene has also been reported to inhibit nodulation on primary and lateral roots in peas *Pisum sativum* (Lee and Larue, 1992). Several crops such as beet root (*Beta vulgaris*), maize (*Zea mays*), wheat (*Triticum aestivum*), barley (*Hordeum vulgare*) sorghum (*Sorghum* sp.) and *Brassica* species

(Chon *et al.*, 2005; Khanh *et al.*, 2005; Oueslati, 2003; Rice, 1984; Roth *et al.*, 2000; Uremis *et al.*, 2005) are known to have allelopathic activity on other crop or weeds. Rizvi *et al.* (1992) reported that allelopathic interactions between crops, whether negative or beneficial, in a crop rotation or mixed cropping system may have direct bearing on the crop.

Sunflower, *Helianthus annuus* has been shown to have allelopathic inhibitory effects on both weed and crop plant species by researchers Anjum *et al.* (2005), Azania *et al.* (2003), Batish *et al.* (2002), Bogatek *et al.* (2005), Ciarka *et al.* (2004) and Gawronska *et al.* (2004). The plant has been reported to be rich in terpenoids, particularly sesquiterpenoids (Macias *et al.*, 1996, 1998, 2002), which have been found to have a wide range of biological activities including potential allelopathy (Harborne, 1993; Macias *et al.*, 1999, 2002). Reduced growth and yield has been reported in some crops planted in sunflower fields (Bogatek *et al.*, 2006).

In Botswana sunflower is sometimes grown intercropped with cereals such as maize and sorghum and in some areas with legumes such as cowpeas, bambara groundnuts and beans. Bambara groundnut (*Vigna subterranea*), is a legume that has been classified as an under-utilized crop plant. It is an important crop in Botswana and contributes significantly to the food security of the country. Bambara groundnuts are well adapted to semi dry conditions such as those found in Botswana. Recently a lot of effort and research has been directed towards the improvement of this crop (Massawe *et al.*, 2003) and some studies have been conducted on the allelopathic activity of some common weeds on bambara groundnut (Karikari *et al.*, 2000). Although the allelopathic potential of sunflower has been reported, the effect on bambara groundnut has not been investigated. Since intercropping between sunflower and bambara groundnut is commonly practiced in Botswana, we determined the allelopathic effects of sunflower or its plant residues on the growth and nodule formation of bambara groundnuts.

## MATERIALS AND METHODS

**Preparations of dry sunflower plant residues:** This study was conducted at the University of Botswana, Department of Biological Sciences, 2002-2003. The sunflower plants, variety Russian No. 4 used in the experiments, were obtained from Botswana College of Agriculture Experimental farms. The plants were grown in the field under irrigation. No fertilizer was applied to the soil throughout the growing period. The plants were harvested after four months, at this time the plants had

already flowered and most of them had mature seeds. Thirty plants were selected randomly from the field and divided into two parts, one part was air dried in outside environment under shade for three weeks and the rest was homogenized to prepare the plant extracts. The air dried plants were then cut into 5 cm pieces and stored in sealed plastic containers and stored at room temperature until required.

**Preparation of aqueous extracts from freshly harvested plants:** The aqueous extract was prepared by extracting 5 g of plant material (root separate from shoots) in 100 mL of distilled water and blended for 15 min using a household blender. The resulting slurry was filtered through four layers of cheese cloth to remove fibre and other impurities. The filtrate was centrifuged at 3000 rpm for 15 min in order to obtain particulate-free supernatant. The supernatant was then filtered through Whatman No. 4 filter paper. The solution was stored at 4°C for use in seed germination experiments.

**Seed germination experiments:** Seeds of bambara groundnuts of uniform size were selected. The seeds were surface sterilized in 10% household bleach solution for 15 min followed by rinsing three times in sterile distilled water. Ten seeds were placed in Petri dishes lined with filter paper. Extracts (15 mL) of sunflower roots, shoots or a combination of roots and shoots, were added to the Petri dishes. Controls containing distilled water were also set up. Dilutions containing 10-90% (v/v) of extracts in distilled water were prepared and tested in seed germination experiments. All germination experiments were replicated four times (to give 40 seeds per treatment). The seeds were incubated at 24°C in the dark. The number of germinated seeds was recorded after five days of incubation; the percent germination was calculated and the root and shoot length measured. The roots were then separated from the shoots, all plant material dried to constant weight in an oven at 80°C for 24 h and the dry weight determined.

## Green house growth experiments

**Mixed planting experiments:** Bambara seeds and sunflower seeds were planted together in 50×20 cm pots containing commercially available potting soil. A total of eight seeds were sown in each pot (4 seeds of bambara groundnuts and 4 of sunflower). Controls pots containing only sunflower or bambara seeds were also prepared. Each treatment was replicated eight times. Plants were watered as and when needed. The plants were allowed to grow for five weeks after which the plants were carefully removed

from the pots. The plant height, number of leaves, number of flowers, number of nodules and dry weights were then determined.

**Sunflower plant residue experiments:** In these experiments, shredded sunflower plant parts were incorporated into the soil in the following ratios, 50:50, 75: 25 (w/w) plant material:soil. No residues were added to the soil in the control treatment. The soil was thoroughly mixed. Eight bambara seeds were planted in each soil mixture and each treatment was replicated four times. After four weeks of growth, ungerminated seed and the bambara seedlings were carefully removed from the pots and the following parameters determined; number of germinated seeds, the plant heights and the numbers of leaves, nodules and flowers.

In order to simulate cultural practices in which bambara nuts are grown in a field from which sunflower has been harvested (with decomposing sunflower plant materials), the soil from above experiment was taken and re-used to grow fresh bambara groundnuts. After four weeks of growth, ungerminated seed and the bambara seedlings were carefully removed from the pots and the number of germinated seeds, the plant heights and the numbers of leaves, nodules and flowers were determined.

Analysis of Variance (ANOVA) was used to analyze the data and difference between means was tested using the Least Significant Difference (LSD) at 95% confidence level.

**RESULTS**

**Effect of sunflower extracts on seed germination and seedling growth of bambara groundnut:** Sunflower extracts (both the shoot and root extracts) inhibited seed germination and seedling growth when compared to the controls (Table 1). Whole plant extracts (mixture of shoot and root extracts) inhibited seed germination more than shoot or root extracts. However, there was no difference between seed germination in shoot and root extracts (Table 1). The growth of the seedlings was also affected by the sunflower extracts; radicle length was reduced more than the shoot length. Sunflower extracts significantly reduced the dry weights of seedlings, with

Table 1: Effect of sunflower aqueous extracts on bambara groundnut seed germination and seedling growth

Treatments	Germination (%)	Shoot Length (cm)	Root Length (cm)	Seedling dry wt. (g)
Control	77.5**	2.9 <sup>a</sup>	5.10 <sup>a</sup>	0.10 <sup>a</sup>
Root extract	50.0 <sup>b</sup>	1.9 <sup>a</sup>	3.20 <sup>b</sup>	0.03 <sup>b</sup>
Shoot extract	35.0 <sup>b</sup>	2.0 <sup>a</sup>	3.00 <sup>b</sup>	0.06 <sup>c</sup>
Mixture	17.5 <sup>c</sup>	2.6 <sup>a</sup>	3.90 <sup>b</sup>	0.03 <sup>b</sup>

\*Within columns means followed by the same letter(s) are not significantly different at p = 0.05 as determined by LSD

the root extracts and the shoot and root extracts mixture showing the greatest effects (Table 1). Diluted extracts also inhibited seed germination and seedling growth of bambara groundnut in a concentration dependent pattern; shoot length was reduced more than radicle length (Table 2).

**Mixed planting experiments:** In these experiments, field conditions were simulated by growing bambara groundnut together with sunflower. No differences was observed in the shoot elongation, number of leaves, nodulation and flowering of bambara groundnut grown together with sunflower and that of bambara groundnuts grown on their own. However there was a significant reduction in dry weight of bambara groundnut when grown mixed with sunflower at 95% confidence level. On the other hand, there was a significant increase in dry weight of sunflower when grown together with the groundnut than when grown on its own (Table 3).

**Effects of sunflower plant residues on the germination and growth of bambara groundnuts:** Sunflower plant residues incorporated in the soil inhibited seed germination, shoot elongation, number of leaves produced and dry weight of bambara groundnuts at different levels of plant residue: Soil mixtures. There was no seed germination recorded in 75:25 plant residue:soil mixture treatments (Table 4). When the amended soil was

Table 2: Effect of sunflower aqueous extracts concentration on bambara groundnut seed germination and seedling growth

Extract conc.	Germination (%)	Shoot length (cm)	Root length (cm)
0	85.00	4.80	6.30
10	65.00	4.00	5.80
20	39.00	3.20	5.70
30	39.00	3.20	5.70
40	29.00	2.60	4.80
50	39.00	2.70	4.80
60	32.00	2.70	4.80
70	41.00	3.40	4.50
80	36.00	3.70	4.50
90	32.00	2.70	4.10
100	32.00	2.60	4.20
SEM	5.14	0.21	0.22

SEM = Standard Error of Means

Table 3: Effect of mixed planting bambara groundnut with sunflower on growth, flowering and nodule formation

Treatments	Plant height (cm)	Leaf No.	Dry weight (g)	Flower No.	Nodule No.
Bambara nut	27.0 <sup>**</sup>	30.0 <sup>a</sup>	3.60 <sup>**</sup>	18.0 <sup>a</sup>	2.0 <sup>a</sup>
Bambara nut ** and Sunflower	23.0 <sup>a</sup>	28.0 <sup>a</sup>	2.98 <sup>b</sup>	19.0 <sup>a</sup>	1.0 <sup>a</sup>
Sunflower only	33.0 <sup>b</sup>	8.0 <sup>b</sup>	2.99 <sup>b</sup>	NA	NA
Sunflower *** and Bambara	32.0 <sup>b</sup>	8.0 <sup>b</sup>	4.75 <sup>c</sup>	NA	NA

\*: Within columns means followed by the same letter(s) are not significantly different at p = 0.05 as determined by LSD. \*\*: Data for bambara groundnut, \*\*\*: Data for sunflower, NA = Not Applicable (plants had not reached flowering stage)

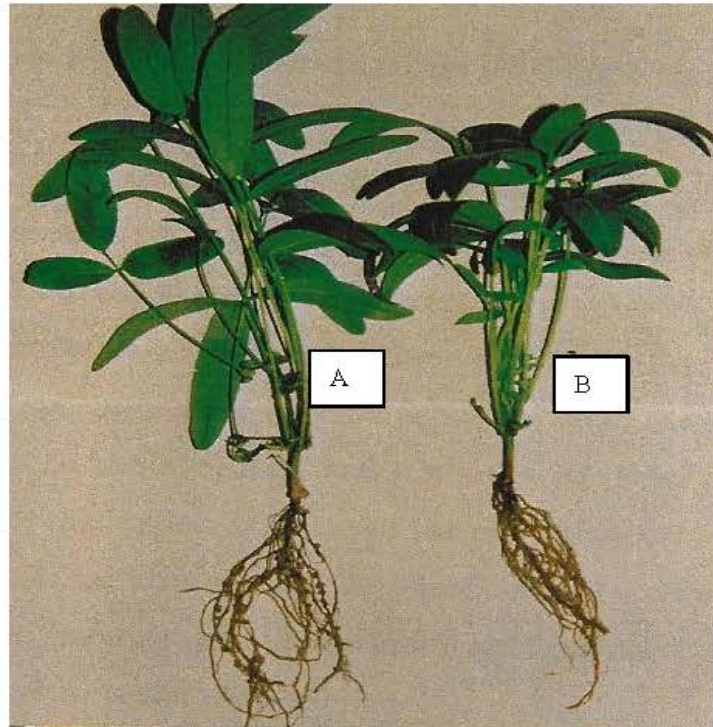


Fig. 1: Effect of sunflower plant residues on the growth and nodulation of bambara groundnut. (A) control and (B) plants grown in soil with residues. Note presence of nodules in the control and their absence in treated plants

Table 4: Effect of sunflower plant residues on bambara groundnut seed germination

Treatments	Exp. 1 (fresh Soil) % germination	Re-used soil % germination
0 Control	84.7**	85.0**
50:50 (plant residue: soil)	13.0 <sup>b</sup>	52.5 <sup>b</sup>
75:25 (plant residue: soil)	0.0 <sup>c</sup>	37.0 <sup>b</sup>

\*. Within columns means followed by the same letter (s) are not significantly different at p = 0.05 as determined by LSD

Table 5: Effect of sunflower plant residues on growth, flowering and nodulation of bambara groundnuts

Treatments	Plant height (cm)	Leaf No.	Dry weight (g)	Flower No.	Nodule No.
Control	19.8**	23.0 <sup>a</sup>	6.96**	7.3 <sup>a</sup>	5.5 <sup>a</sup>
50:50	16.0 <sup>b</sup>	15.3 <sup>b</sup>	3.70 <sup>b</sup>	0.0 <sup>b</sup>	0.0 <sup>b</sup>
75:25	0.0 <sup>c</sup>	0.0 <sup>c</sup>	0.00	0.0 <sup>b</sup>	0.0 <sup>b</sup>

\*. Within columns means followed by the same letter(s) are not significantly different at p = 0.05 as determined by LSD

re-used in the second experiment, there was an increase in seed germination of groundnut at both levels of soil incorporation; however this was significantly lower than that observed in the controls (Table 4). Sunflower residues also completely inhibited flowering and nodulation within the experimental period, while plant height was significantly reduced (Table 5, 6 and Fig. 1).

Table 6: Effect of decomposed sunflower plant residues (re-used soil) on growth, flowering and nodulation of bambara groundnut

Treatments	Plant height (cm)	Leaf No.	Flower No.	Nodule No.
Control	20.0**	28.0 <sup>a</sup>	6.0 <sup>a</sup>	33.8 <sup>a</sup>
50:50	17.0 <sup>b</sup>	27.0 <sup>b</sup>	0.0 <sup>b</sup>	0.0 <sup>b</sup>
75:25	16.0 <sup>b</sup>	25.0 <sup>b</sup>	0.0 <sup>b</sup>	0.0 <sup>b</sup>

\*. Within columns means followed by the same letter(s) are not significantly different at p = 0.05 as determined by LSD

## DISCUSSION

Allelopathic phenomenon has received much attention as shown by the numerous reports on the subject (Anaya, 1999; Harper *et al.*, 2005; Khanh *et al.*, 2005; Narwal *et al.*, 1998; Reigosa *et al.*, 2006; Singh *et al.*, 2001; Weston and Duke, 2003). Present study has shown that sunflower (*Helianthus annuus*) extracts or its residues incorporated into soil affect seed germination, growth and development and nodulation of the bambara groundnut, *Vigna subterranea*. Laboratory experiments showed that aqueous extracts of sunflower contained substances that inhibited seed germination and seedling growth of the groundnuts. Root and shoot length and dry

matter accumulation were reduced, with the root being affected more than the shoot. The inhibition of seed germination and seedling growth was concentration dependent. Present results are similar to those obtained by other workers who observed inhibition of seed germination and seedling growth on other crops and weeds by sunflower extracts (Batish *et al.*, 2002; Bogatek *et al.*, 2005; Anjum *et al.* 2005). The allelochemicals inhibit germination and seedling growth probably by affecting cell division and elongation, processes that are very important at this stage, or by interfering with enzymes involved in the mobilization of nutrients necessary for germination. Levizou *et al.* (2002) found low mitotic index in root apex of lettuce treated with leaf extracts from *Dittrichia viscosa*.

The results of mixed planting sunflower with bambara groundnut showed a significant reduction in dry matter accumulation, while plant elongation, leaf production, flowering and nodule formation in bambara groundnuts was not affected. However, sunflower appeared to have benefited from the presence of bambara groundnut in this system (Table 2). A significant increase in dry weight of sunflower was obtained. This could have been due to increased availability of ammonia through nitrogen fixation activity of the bambara groundnut. Present results further support the known beneficial effect of nitrogen fixation by legumes to other plants. Intercropping of crops with legumes is a common practice in Botswana and many other countries due to realization of the benefits obtained from biological nitrogen fixation by legumes. This work has shown that intercropping sunflower with bambara groundnuts had some beneficial effect on the sunflower and did not have negative effects on the development of bambara groundnuts; seed germination, plant elongation, flowering and nodulation were not affected. However, these results should be interpreted with caution in view of other reports that indicate strong allelopathic activity of sunflower on other plants (Batish *et al.*, 2002).

Incorporating sunflower residues in the soil highly affected several developmental processes in bambara groundnuts. Seed germination, leaf formation and shoot elongation were reduced. The effect increased with increased amount of residues incorporated into the soil and flowering and nodulation were completely inhibited during the experimental period (five weeks). When the same soil was re-used in a subsequent experiment in order to simulate traditional farming practices there was an improvement in bambara seed germination but flowering and nodulation were completely inhibited. It is important to note that although bambara groundnut plants were generally smaller in the re-used soil than in the first experiment, more leaves and nodules were produced in the

control plants (34 vs 6) probably as a response to nutrient (nitrates) deficiency in the soil. The inhibitory effects of sunflower on weeds and crop plants species have also been reported by many researchers both in the laboratory and under field conditions (Anjum *et al.*, 2005; Azamia *et al.*, 2003; Batish *et al.*, 2002; Bogatek *et al.*, 2006; Ciarka *et al.*, 2004). Present study has also confirmed inhibitory effects of sunflower extracts or its residues on seed germination and growth and nodulation of bambara groundnut. The results are quite significant in view of the intercropping between sunflower and bambara groundnut that is practiced in some parts of Botswana. The results indicate that sunflower contained allelochemicals that, when released in the soil through decomposition and leaching can affect growth and development of bambara groundnut including flowering and nodulation. Flowering and nodulation are important developmental processes which affect plant yield.

Legumes contribute significantly to soil fertility through biological fixation of atmospheric nitrogen to ammonia which can be used by the plant. This is possible through a symbiotic relationship between rhizobia and leguminous plant which lead to the formation of root nodules. This study has shown that sunflower residues strongly affected the development of nodules in bambara groundnut. Nodule development involves the interaction between rhizobia and legume roots (Limpens and Bisseling, 2003; Schulze and Kondorosi, 1998). The development of a root nodule is under the control of nodulation (nod) genes. Legume roots secrete flavonoids which act as signals to activate the expression of nod genes resulting in the production of rhizobial NOD factors that induce root cortical cells to divide and develop into nodules (Schulze and Kondorosi, 1998; Spaink, 2000). The curling and deformation of root hairs are considered to be the early signs of the beginning of nodulation due to rhizobial bacteria infections. Allelochemicals from sunflower may have suppressed any of these processes. Levizou *et al.* (2002) has reported inhibition of root hair development by allelochemicals. Sunflower has been found to be rich in terpenoids particularly sesquiterpenoids (Marcias *et al.*, 2002) and these compounds have been reported to have a wide spectrum of biological activity including allelopathic properties. High amounts of these compounds may have been released into the soil through decomposition of the plant residues thereby inhibiting nodulation.

The present results have shown that sunflower produced substances which adversely affected the development of bambara groundnuts by inhibiting germination, seedling growth, flowering and nodulation.

Inhibition of these processes have direct effect on yield in crops. In most traditional farming practices, plant remains from a previous crop are usually incorporated into the soil and allowed to decompose in order to improve the soil. During the following season, the field is then either planted with the same crop or a different crop in some kind of crop rotation. However, incorporating sunflower plant parts in the soils with a view of improving soil organic components and soil texture as is normally practiced by farmers in Botswana should be avoided. The plant remains decompose and release into the soil, substances that can be phytotoxic (Batish *et al.*, 2002). Therefore careful considerations should be given to the choice of crops for intercropping or rotations, especially in rotations. It is also recommended that sunflower plant parts should be removed from the farms in order to minimize their detrimental effect on subsequent crops.

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