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Allelopathic potential of mexican sunflower [tithonia diversifolia (hemsl) a. Gray] on germination and growth of cowpea seedlings (*vigna sinensis* l.)

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A B S T R A C T

Allelopathy is a common among plants. Allelopathic substances affect seed germination, plant growth and development or even affect chlorophyll synthesis by the plant leaves. Tithonia diversifolia contains allelochemicals that inhibit growth of many agricultural crops. A study was conducted at Maseno University (Kenya) to investigate the allelopathic effects of T. diversifolia on the germination of seeds, growth and synthesis of chlorophyll of seedlings of cowpea (Vigna sinensis). Cowpeas seeds and seedlings were subjected to four different concentrations of the fresh shoot aqueous extracts which comprised of 0 (tap water), 25%, 50%, 75%, and 100%. Twenty Seeds of cowpeas were germinated in sterilized petri dishes lined with a layer of filter paper whatman no. 1, moistened with 10ml of each extract concentration and tap water respectively. Germination percentage, plumule and coleoptiles length were determined at the end of the study. Individual cowpeas seedlings were planted in 4.5 litres plastic pots containing soil and irrigated with the various shoot extracts concentrations and tap water using 500ml respectively. The treatments were replicated five times and the pots laid out as completely randomized design in the glasshouse. Data on shoot height; number of leaves per plant was recorded on weekly basis. Leaf area, shoot and root dry weights and leaf total chlorophyll, chlorophyll a and b concentration were determined at the end of the experiment. Data collected was subjected to analysis of variance (ANOVA) using SAS statistical package. Treatment means were compared using the least significant difference (LSD) at ($P<0.05$). The results revealed that fresh shoot aqueous extracts of T. diversifolia have both inhibitory and stimulatory effects on V. sinensis. Seed germination increased with increasing concentration of shoot extracts from 25%, 50% to 100% even though there were no significance differences among treatments. Plumule and radicle lengths reduced significantly among treatments. Shoot heights, leaf number, leaf area, shoot and root dry weights, total chlorophyll and chlorophyll a and b concentrations increased significantly with increasing shoot extract except at 100% treatment where all these parameters were inhibited.

Key words: *allelopathy, Cowpeas, germination, growth, radicle growth*

Introduction

Invasive plant species when introduced into new environments may result into a number of ecological problems (Howard et al., 2000).They may influence other species through pathways such as allelopathy or phytotoxicity (Samuel et al., 2005; Imeokpara and Okusanya, 1994; Ayeni et al., 1997). Allelopathy is the inhibitory or stimulatory effect of a plant on another by chemicals released from the donor plant to the environment (Taiwo and Makinde, 2005; Bano et al., 2012). Allelopathy is a process by which plants release chemical compounds in their vicinity (Rice, 1984). Studies have shown that different plant parts release toxic metabolites into the soil that affect adversely germination and growth of plants (Kumar et al., 2008). The importance of allelochemicals in agro-ecosystem has attracted the attention of numerous scientists (Otusanya and Ilori, 2014; Pukclai and Kato-Noguchi, 2012).

In an allelopathic study of native species on a major plant invader in Europe Christina et al. (2015) indicated that by day 50, shoot height had decreased by 27% for plants originating from rhizomes. Tithonia diversifolia has both growthinhibiting and growth-stimulating properties (Chukwuka et al., 2014; Ademiluyi, 2012; Ilori et al., 2007). Tongma et al. (1998) have reported the allelopathic effect of T. diversifolia on shoot and root growth. Oyerinde et al. (2009) have shown that Tithonia diversifolia has no effect on germination of Zea mays, but can inhibit radical and the plumule growth in germinating seeds. Allelopathic compounds in plant extracts may decrease cell turgor, growth and photosynthetic rate of plants (Yarnia et al., 2009; Singh et al., 2010).

Cowpea (Vigna sinensis) plays an important role in many communities in Africa (Singh, 2002; Langyintuo et al., 2003) such as in human nutrition (Ahenkora et al., 1998; Muthamia and Kanampiu, 1996; Singh et al., 1997; Saidi et al., 2010). Cowpeas protein content is about 25% (Ndakidemi and Dakora, 2007). Studies indicate that T. diversifolia is allelopathic to some food crops (Taiwo and Makinde, 2005). The main objective of this study was to investigate the allelopathic effects of Mexican sunflower on germination of cowpea seeds, growth and chlorophyll synthesis of seedlings of cowpeas (V. sinensis). It was hypothesized that T. diversifolia aqueous shoot extracts inhibit germination of seeds, growth and chlorophyll synthesis of cowpeas.

Materials And Methods

Collection of Plant Materials

Collection of T. diversifolia was from the field near botanic garden at Maseno University, Kenya. Cowpea seeds obtained from Muguga seed bank.

Preparation of Fresh Shoot aqueous Extracts of T. diversifolia

One hundred and eight (108) grams of fresh shoots of T. diversifolia were harvested at vegetative stage and cut into small pieces of about 4cm length. The small pieces were finely ground with pestle and mortar, and then soaked in 1 litre of tap water in a large beaker for 24hrs. The collected extracts were filtered through cheese cloth to remove debris and finally filtered using Whatman No. 1 filter paper to have 100% concentration. Aqueous extracts of 25%, 50% and 75% concentrations were made by diluting the original extract with distilled water according to Musyimi et al. (2012).

Germination Experiment

Germination tests were carried out in a glasshouse at the Maseno University Botanic garden. Twenty (20) uniform seeds of cowpeas were soaked in 5% Sodium hypochlorite to prevent fungal infection. They were then rinsed for about 5 minutes in running water. The seeds were then be washed in distilled water and then placed in dried Petri dishes lined with layers of Whatman No. 1 filter papers and moistened with 10ml of the respective aqueous extracts(treatment) and distilled water(control). The treatments were replicated five times. Data on seeds germinating each day were recorded and germination percentage calculated after three weeks of treatment. Radicle and plumule length of the seedlings were measured using transparent meter rule upto the end of the experiment.

Growth Experiment

Growth tests were carried out in a glasshouse in the University Botanic garden. Twenty five (25) four and half (4.5) Litrer plastic pots were filled with 2kg of humus soils collected from Botanic garden which had been solarised (sun sterilized) for at least two days to prevent fungal growth. The Maseno soils are classified as acrisol, deep reddish brown friable clay with pH ranging from 4.5 to 5.5, soil organic carbon and phosphorus contents are 1.8% and 4.5mg kg^{-1} respectively (Netondo, 1999).The pots were perforated at the bottom to avoid water logging. Ten seeds of Vigna sinensis were sown in each of the pots. Watering was done every morning with 500ml tap water per pot to the end of two weeks. After two weeks, the seedlings in each pot were thinned down to two plants per pot. The pots were allocated to control (tap water) and four different treatments (25%, 50%, 75% and 100% extracts concentrations) and they were laid out in completely randomized design. Thereafter, the control pots were supplied with 500ml tap water daily and the pots with the different aqueous treatments were supplied with 500ml of the appropriate aqueous extract daily. The seedlings were maintained in the glasshouse under natural sunlight, relative humidity 70-80 %, photosynthetically active radiation 500-700 µmol m^2s^{-1}; average day temperatures were 23 ± 2^0C. Data collection commenced before and after the initiation of the shoot extracts treatments.

Measurement Of Parameters

Determination of shoot height

Shoot height was measured from the soil level to the upper point of the terminal bud of the seedling using a meter rule, every week up to the end of the experiment.

Determination of leaf number

Number of fully expanded mature leaves per plant were counted and recorded on each plant every week up to the end of the experiment.

Determination of leaf area

Leaf length and leaf width used to calculate leaf area was measured using a meter rule. Leaf area was then calculated using the formula of Otusunya et al. (2007b), as shown below:
LA= 0.5(L X W) where, L= Length of leaf and W= Maximum width.

Determination of root and shoot fresh and dry weights

At the end of the experiment, the plants were carefully uprooted from the soil, cleared off the attached soil, separated into root and shoot and then measured separately using an electronic weighing balance. Fresh plants (roots and shoots) were packaged separately in envelopes and dried to constant weight at 80^0c in an oven. Root and shoot dry weights were determined on an electronic weighing balance, and then mean weights calculated.

Determination of leaf chlorophyll concentration

Determination of chlorophyll concentration followed the formula of Adelusi et al. (2006). The fourth fully expanded leaf from shoot apex was sampled from all the treatments. 0.5g of these leaves were grounded in 20ml of 80 %(v/v) acetone using mortar and pestle. The resulting substrate was read at 664 and 647 nm using UV- Visible Spectrophotometer. Chlorophyll a, b and total chlorophyll concentration were calculated as follows:
Chlorophyll a= 13.19 A664-2.57 A647 (mg g^{-1} fresh weight).
Chlorophyll b= 22.1 A647- 5.26 A664 (mg g^{-1} fresh weight).
Total chlorophyll = 7.93 A664+ 19.53 A647 (mg g^{-1} fresh weight).
where, A664 is the absorbance at 664nm A647 is the absorbance at 647 nm.

Data analysis

Data obtained from the study was subjected to analysis of variance (ANOVA) in SAS statistical package. Treatment means were separated and compared using Least Significance Difference (LSD at 0.05).

RESULTS

Effects of fresh shoot aqueous extracts of T. diversifolia on germination and growth of plumule and radicle of cowpeas

Aqueous shoot extracts of T. diversifolia increased seed germination percentage of cowpeas by 8% while at the same time reducing significantly ($P<0.05$) the growth of the plumule and radicle (Table 1). The plumule length was reduced by 16% while the radicle length was reduced by 64% respectively.

Table 1. Effects of fresh Shoot aqueous extracts of T. diversifolia on germination percentages, plumule lengths and radical lengths of cowpeas.

Extract concentration (%)	Germination %	Plumule lengths (cm)	Radicle lengths (cm)
0	60a	3.53a	5.25a
25	61a	3.35ba	3.36b
50	63a	3.23bac	2.64c
75	64a	3.15bc	2.35c
100	65a	2.98c	1.88d

Means followed by the same letters within each column are not significantly different according to LSD ($p < 0.05$). (0) control, 25, 50, 75 and 100 denote the concentration of extract of T. diversifolia.

Effects of fresh shoot aqueous extracts of T. diversifolia on shoot heights of cowpeas seedlings.

Shoot heights increased significantly ($P<0.05$) with increasing shoot extracts of T. diversifolia in all the treatments except at 100% treatment (Table 2). The increase in shoot height was about 109% of the control seedlings by week 3.

Table 2. Effects of fresh shoot aqueous extracts of T. diversifolia on shoot heights of cowpeas seedlings.

Extract concentration(%)	Week1	Week2	Week3
0	16.20d	37.70d	53.40c
25	17.40bc	41.00c	55.40b
50	17.90b	42.60b	57.60a
75	18.36a	44.80a	58.40a
100	17.20c	40.80c	53.40c

Means followed by the same letters within each column are not significantly different according to LSD ($p < 0.05$). (0) control, 25, 50, 75 and 100 denote the concentration of extract of T. diversifolia.

Effects of fresh shoot aqueous extracts of T. diversifolia on number of leaves of cowpeas seedlings

Number of leaves increased significantly (P<0.05) with increasing concentration of the shoot extracts in all the weeks (Table 3). However there was a reduction in number of leaves at the highest extract concentration (100%). Aqueous shoot extracts increased leaf number by 41% by the third week.

Table 3. Effects of fresh shoot aqueous extracts of T. diversifolia on number of leaves of cowpeas seedlings.

Extract concentration (%)	Week1	Week 2	Week3
0	4.2d	7.6cd	8.8c
25	5.2c	8.0bc	9.6c
50	5.8b	8.4b	12.0b
75	6.6a	9.6a	13.2a
100	5.4bc	7.0d	12.4b

Means followed by the same letters within each column are not significantly different according to LSD ($p < 0.05$). (0) control, 25, 50, 75 and 100 denote the concentration of extract of T. diversifolia.

Effects of fresh shoot aqueous extracts of T. diversifolia on leaf area of cowpeas seedlings

Leaf area significantly (P<0.05) increased with increasing concentration of aqueous shoot extracts of T. diversifolia in the 3 weeks of treatments, except at 100% where leaf area was inhibited (Table 4). By week three leaf areas had increased by 95% of the control treatment.

Table 4. Effects of fresh shoot aqueous extracts of T. diversifolia on leaf area of cowpeas seedlings.

Extract concentration (%)	Week 1	Week 2	Week 3
0	0.75e	1.99d	3.18e
25	0.99b	2.62c	4.24c
50	1.32b	3.31b	5.36b
75	1.64a	4.41a	6.19a
100	0.83d	2.38c	3.58d

Means followed by the same letters within each column are not significantly different according to LSD ($p < 0.05$). (0) control, 25, 50, 75 and 100 denote the concentration of extract of Tithonia diversifolia.

Effects of fresh shoot aqueous extracts of T. diversifolia on dry weights of shoots and roots of cowpeas seedlings.

There was significant (P<0.05) difference in dry weight of shoots and roots among treatments in weeks 1, 2 and 3 (Table 5). The highest fresh and dry weight measurements were obtained from roots of plants supplied with 75% of aqueous extracts of T. diversifolia treatment. There was about 44% and 144% increase in shoot and root dry weights respectively in comparison to control after 3 weeks of aqueous shoot extract treatments.

Table 5. Effects of fresh shoot aqueous extracts of T. diversifolia on dry weights of shoots and roots of cowpeas seedlings.

Extract concentration (%)	Dry weight of Shoots	Dry weight of Roots
0	3.48c	0.16d
25	3.66cb	0.24c
50	4.50ab	0.33ab
75	5.02a	0.39a
100	3.78bc	0.31bc

Means followed by the same letters within each column are not significantly different according to LSD ($p < 0.05$). (0) control, 25, 50, 75 and 100 denote the concentration of extract of Tithonia diversifolia.

Effects of fresh shoot aqueous extracts of T. diversifolia on total chlorophyll, chlorophyll a and b of cowpeas seedlings.

Total leaf chlorophyll content, chlorophyll a and b were increased by treatment with aqueous shoot extracts of T. diversifolia (Table 6). Total chlorophyll increased by 153% of the control treatment. Chlorophyll a and b increased by 79% and 423 % of the control treatment. There were significant (P<0.05) differences between seedlings treated with aqueous extracts and control seedlings.

Table 6. Effects fresh shoot aqueous extracts of T. diversifolia on chlorophyll a, b and total chlorophyll of cowpeas seedlings.

Extract concentration (%)	Chl a	Chl b	Total Chl
0	0.53b	0.13b	0.66b
25	0.96a	0.59a	1.55a
50	0.98a	0.61a	1.59a
75	0.99a	0.68a	1.67a
100	0.95a	0.63a	1.58a

Means followed by the same letters within each column are not significantly different according to LSD ($p < 0.05$). (0) control, 25, 50, 75 and 100 denote the concentration of extract of T. diversifolia.

Discussion

The aqueous shoot extracts of T. diversifolia affected the germination of cowpeas seeds (Table 1). The results indicated that shoot extracts are desirable in enhancing germination of cowpeas. Increase in germination and growth of the cowpeas seedlings may be due to action by some allelochemicals that are involved in cell division. For instance, Ilori et al. (2007) reported stimulatory effect of T. diversifolia on the germination and growth of O. sativa. Contrary to our results, previous allelopathic studies have reported reduction in seed germination and seedling growth (Zoheir et al., 2008; Ferguson and Rathinasabapath, 2009). The radicle and plumule lengths of V. sinensis were both significantly inhibited (Table 1) as the concentration of plant extract increased. Similar findings have been reported by Abu–Roman et al. (2010) while studying the allelopathic effect of Spurge (Euphorbia hierosolymitana) on wheat (Triticum durum) seedlings. In this study, radicle length appeared to be more sensitive to shoot extracts than the plumule length. These results are in agreement with the finding that water extracts of allelopathic plants inhibit radicle growth more than hypocotyls growth (Ashrafi et al., 2007).

Our results are also in agreements with the findings of Hussain et al. (1985) who reported that Azadirachta indica leaf extract reduced radical growth of several crops. Allelochemicals may reduce cell division hence reducing plumule and radicle elongation. For instance, inhibition activity of phytochemicals such as p –coumaric, and ferulic acids and volatile constituents of essential oils from plants families such as the Asteraceae family where T. diversifolia belong are known (Vyvyan, 2002). Otusanya et al. (2007b) demonstrated that aqueous extract and shoot extract of T. diversifolia are inhibitory to the germination and growth of Amaranthus cruentus.

From this study growth, that is, shoot height, leaf number, leaf area, shoot and root dry weight and leaf area of cowpea seedlings treated with aqueous extract of T. diversifolia were observed to be significantly stimulated to grow compared to the control plants at the end of the three weeks experimental period (Tables 2, 3, 4 and 5). The leaves of the aqueous extract irrigated plants were healthier and more green compared to the control plants. Possibly the plants irrigated with the aqueous extracts of T, diversifolia accumulated more materials for development which was reflected in the shoot height, leaf number, leaf area and shoot and root dry weights increments as opposed to control seedlings, suggesting that cowpeas under study could adapt to the environment of T. diversifolia allelopathic influence with a little harm. A similar effect was reported in wheat seedlings by Hussain et al. (2007). The results in this research also confirm observation made by Musyimi et al. (2012). Aladejimokun et al. (2014) and Ademiluyi (2012) indicated that allelochemicals in T. diversifolia extracts stimulated plant growth of some plants.

Biomass production and distribution can be important mechanisms by which plant species can survive under environmental stress. In this study, T. diversifolia leaf extracts stimulated shoot and root dry weights. These findings are in agreement with previous results by Bano et al. (2012), where neem leaf extracts significantly stimulated root growth of Wild oat seedlings. Increase in leaf area may lead to increased surface area for light harvesting hence increased photosynthesis and increased biomass gain and shoot growth. These findings indicate a maximum carbon – fixation adaptation mechanism in this cowpeas variety in relation to allelopathy. At high shoot extracts concentration (100% shoot extracts) growth parameters were inhibited, this may be due to inhibition of cell division and elongation in the plant parts (Zhang and Fu, 2009). The response of allelochemicals may be concentration dependent and inhibit the growth of some species at certain concentrations or even stimulate the growth of the same or different species at different concentrations (Azania et al., 2003).

There were significant differences in total chlorophyll, chlorophyll a and b concentration in leaves of cowpeas seedlings treated with shoot extracts of T. diversifolia compared to control seedlings (Table 6), and this could be probably due to the presence of nutrients such as nitrogen and magnesium in the shoot extracts. Similar findings have been reported by Musyimi et al. (2014), using the leaf green biomass of S. sesban on S. nigrum L. var. popolo plants. Studies by Mohammadi et al. (2010) showed that leafy green biomass significantly increased leaf chlorophyll of chickpea. Tithonia diversifolia is a high quality organic material in terms of nutrient release and supplying capacity (Ojeniyi et al., 2012). Jama et al. (2000), Nziguheba et al. (2002) and Sangakkara et al. (2002) show that green biomass of T. diversifolia can serve as an effective source of nutrients for crops.

It is possible that supply of Nitrogen, iron and magnesium elements by the shoot extracts contributed to the increase in total leaf chlorophyll, chlorophyll a and b concentration at lower extracts concentrations. In contrast to our findings Elisante et al. (2013) found that in legume plants, the total chlorophyll content was ultimately affected and its accumulation was significantly reduced in plants treated with both aqueous seed and leaf extracts of Datura stramonium. Also contrary to our results chlorophyll concentration was reduced by fresh shoot extracts of T. diversifolia on seedlings of Zea mays (Oyerinde et al., 2009). Reduction in chlorophyll content at higher shoot extract concentration (100% shoot extract) implies impaired or reduced photosynthesis, thereby leading to a reduction in growth of the plants as evidenced with various growth parameters at high shoot extracts concentration (Yang et al., 2002).

Conclusion

The results indicate that fresh shoot aqueous extracts of Tithonia diversifolia contain allelochemicals which significantly affect germination and growth of cowpeas (Vigna sinensis). Fresh shoot aqueous extracts of T. diversifolia stimulated cowpea seed germination percentage. The plumule and radicle length were inhibited by the shoot extracts of tithonia diversifolia. The shoot extracts stimulated shoot height, number of leaves, leaf area, shoot and root dry weights of Cowpea seedlings. The aqueous shoot extracts influenced the synthesis of chlorophyll as evidenced by the increase in total chlorophyll, chlorophyll a and b concentration at lower extracts concentrations. These results confirm both beneficial and inhibitory functions of the allelochemicals in T. diversifolia aqueous shoot extracts. Therefore, T. diversifolia can be recommended to be used as leafy biomass in farms growing cowpeas (Vigna sinensis). This will improve their growth and productivity.

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