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Influence of altered temperatures on allelopatic properties of *Amaranthus cruentus* L.

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ABSTRACT

The relationships between allelochemicals and environmental factors are a key factor for the growth of plants under rotation. We investigated the allelopathic potential of *Amaranthus cruentus* L, grown under different temperature conditions in *in vitro* bioassays. An inhibitory effect on germination and growth of lettuce (*Lactuca sativa* L.), tomato (*Solanum lycopersicum* L.), pepper (*Capsicum annuum* L.) and cucumber (*Cucumis sativus* L.) was observed when seeds were subjected to the leaf litter of *Amaranthus cruentus*. Analysis from our study indicated that germination percentage was significantly affected by growth temperatures (T) of the amaranth ($P < 0.0001$), litter concentration (C) ($P < 0.0001$), vegetable type (V) ($P < 0.0001$), the T × V interaction ($P = 0.0041$) and V × C interaction ($P < 0.0001$). Pepper was the most sensitive with a decline in germination percentage at increasing concentrations (0, 1 and 5 mg ml^{-1}) of litter. Hypocotyl and seminal root lengths were adversely influenced by the plant litter for all the temperature treatments, although effects were most severe when exposed to the leaf litter of the hot temperature treatment. The inhibition caused by the litter was dependent on growth temperature and concentration, while each vegetable species showed different levels of sensitivity.

Key words: *Amaranthus cruentus*; allelopathy; vegetables; germination; hypocotyl; seminal root; crop rotation

IZVLEČEK

VPLIV SPREMENJENIH TEMPERATUR NA ALELOPATSKE LASTNOSTI MEHIŠKEGA ZRNATEGA ŠČIRA *Amaranthus cruentus* L.

Odnosi med alelokemikalijami in okoljskimi dejavniki so odločilni za rast rastlin v kolobarju. V razmerah in vitro poskusa je bil preučevan alelopatski potencial mehiškega zrnatega ščira (*Amaranthus cruentus* L.) v različnih temperaturnih razmerah. Inhibitorni učinek na kalitev in rast vrtne solate (*Lactuca sativa* L.), paradižnika (*Solanum lycopersicum* L.), paprike (*Capsicum annuum* L.) in kumar (*Cucumis sativus* L.) je bil ugotovljen, kadar so bila semena teh vrtnin izpostavljena listnim ostankom mehiškega zrnatega ščira. Analize so pokazale, da so na odstotek kalitve značilno vplivali rastna temperatura (T) ščira (T) ($P < 0.0001$), količina listnih ostankov (C) ($P < 0.0001$) in vrsta vrtnine (V) ($P < 0.0001$) kot tudi interakciji T × V ($P = 0.0041$) in V × C ($P < 0.0001$). Najbolj občutljiva je bila paprika z upadom odstotka kalitve pri naraščanju koncentracije ščira (0, 1 in 5 mg ml^{-1}). Na dolžino hipokotila in semenske korenine so ostanki ščira vplivali negativno pri vseh temperaturah, vendar so bili učinki izrazitejši pri visokih temperaturah. Inhibicija, ki so jo povzročili ostanki ščira, je bila odvisna od njihovih količin in rastnih temperatur, vendar je vsaka od vrtnin pokazala različno občutljivost.

Ključne besede: *Amaranthus cruentus;* alelopatija; vrtnine; kalitev; hipokotil, semenska korenina; setveni kolobar

1 INTORODUCTION

Amaranthus cruentus L., also known as “Mexican grain amaranth” belongs to the family Amaranthaceae and is characterised by being an annual, pseudo-cereal with broad leaves which are used as a leafy vegetable and a forage crop (Saunders & Becker, 1984; Steckel, 2004; Yaacob et al., 2004.). *Amaranthus* spp. have been traditionally used in medicine for the treatment of various diseases and their antioxidant properties have been recorded by various authors (Olumayokun et al., 2004; Dhellot et al., 2006; Mensah et al., 2008). The habitat where amaranth is found differs dramatically and the temperatures that they can grow ranges from 20-

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35 °C (Grosz-Heilman et al., 1990; Guo & Al-Khatib, 2003). Given its several advantages as a crop, its unique nutritional properties, and its use as food and feed (Ayo, 2001; Bavec & Bavec, 2006; Grobelnik et al., 2009), grain amaranth is receiving increasing attention as an alternative crop worldwide. *Amaranthus cruentus* is one of three amaranthus species cultivated as vegetable and grain source. The other two are *Amaranthus caudatus* L. and *Amaranthus hypochondriacus* L. (Olaniyi, 2007; Olaniyi et al., 2008). Recently, Mlakar et al. (2012) compared the allelopathic activities of extracts prepared from fresh roots, stems, leaves, and inflorescence with seeds of weedy and grain amaranths. It was found that at varying concentrations, the aqueous extracts of the grain amaranth, *A. cruentus*, exert allelopathic activity. Compared to the pigweed amaranth, the grain species displayed a stronger inhibitory effect on the germination process, and root elongation of garden cress. Allelopathy can affect a whole range of aspects in agroecosystems namely, weed management, plant reproduction, species associations, the mulching effect on crops and the succession and rotation of cultivated species (Chon et al., 2006; Rawat et al., 2017). These results point out the problematic consequence when amaranth and more specific, *A. cruentus*, is cultivated in crop rotation systems. Understanding this biological occurrence could help to improve applications in both natural and agricultural systems and is helpful for planning and managing cropping systems (Gronle et al., 2015). Furthermore, climate change influences a plant's chemical response and the ecological function of plant allelochemicals (Harvey & Malcicka, 2015). Sudden changes in temperature may influence the production of chemical compounds and the allelopathic properties of a plant (Maqbool et al., 2013). The allelopathic potential of *Amaranthus retroflexus* increased when grown at high latitudes compared to plants grown at low latitudes, indicating the role of temperature on the expression of allelochemicals (Wang et al., 2017). Therefore, the aim of this study was to investigate the relationship between temperature variation and allelopathic effects of different concentrations of plant litter of *A. cruentus* on the germination and growth of some vegetable species in order to determine the crops' feasibility for intercropping and in rotation systems.

2 MATERIALS AND METHODS

2.1 Plant material

Amaranthus cruentus 'Anna' seeds were planted in pots containing a soil-compost (80:20 v/v) mixture and grown at 28/21 °C; day/night temperatures in climate controlled chambers at The Department of Agriculture, University of the Free State. Fifty pots per chamber with an average of three plants per pot were kept in three different chambers and daylight was set at 12 hours in order to prevent flowering. The plants were watered every second day and fertilised with Nutrifeed solution (Starke Ayres). After three months plants were subjected to cool (14/7 °C) and hot (33/40 °C) temperatures. Stress continued for 14 days where after aerial parts of the plants were harvested and the plant material lyophilised, ground into a fine powder and kept at 4 °C until further analyses. Optimum (28/21 °C) temperature grown plants serve as temperature controls.

2.2 Vegetable seeds

Seeds were obtained from Starke Ayres: Tomato 'Money Maker', Sweet Pepper 'California Wonder', Cucumber v 'Ashley' and Lettuce 'Great Lakes'.

2.3 Allelopathy bioassay

The 'sandwich method' of Fujii et al. (2003) was used for determining the *in vitro* phytotoxicity of the leaf litter from the different temperature treatments of *A. cruentus* on the various vegetable seeds. For this method a 5 ml layer of 0.5 % (w/v) sterile water agar was poured into each well of a sterile multi-dish plate and allowed to set. The *A. cruentus* leaf litter was placed on this bottom layer and a second layer of 5 ml sterile water agar was added on top. This made a sandwich of powdered leaf litter between the two layers of agar (10 ml in total). This method was used in order to physically separate the *A. cruentus* samples from the tested seeds, however allows for diffusion of any active component from the sample through the barrier agar layer. The phytotoxicity of 10 and 50 mg of the powdered leaf litter was measured (1 and 5 mg ml^{-1} litter per well) against the different vegetable seeds. Controls contain no leaf material.

Vegetable seeds were surface sterilised by washing in 96 % ethanol for 1 minute followed by 1.30 min in 3.5 % NaCl and finally back into ethanol for 30 seconds. After sterilization the seeds were placed on sterile filter paper and left to dry within a laminar flow cabinet (Labotec, airflow from above). The seeds were then positioned vertically on the top layer of agar in each well, plates were closed and incubated at 25 °C in black bags (full darkness) for 3-7 days depending on the vegetable used. On the 3^{rd} to 7^{th} day the germination percentage and length of the seminal root and hypocotyl was measured. Each of the experiments was done in triplicate and presented as the mean of the replicates.

2.4 Statistical analysis

The results were expressed as means with least significant difference (*LSD*). Analysis of variance (ANOVA) was performed using SAS 9.3 (Institute Inc., Cary, NC, USA, 2008) statistical programme for data and Tukey-Kramer's *LSD* procedure for comparison of means. Significance of differences compared to the control groups was determined using the t-test (Steel & Torrie, 1980).

3 RESULTS AND DISCUSSION

A. cruentus is used as a food crop, and both its grain and leaves being consumed. This plant is now being considered as a new agricultural crop; it is therefore important to understand how this plant will interact within a changing environment and how this will influence other plants. In addition to the increase in average temperatures, global warming is also characterized by an increase in the frequency of the occurrence of extremely high and low temperatures (Wagner, 1996; IPCC 2007). This study showed that allelopathic activity of *A. cruentus* could be affected by environmental temperatures, which can affect other crops negatively in rotation systems. Highly significant (P < 0.01) differences in germination percentage of the various vegetables exposed to the litter of amaranth plants grown under stress or optimal temperature conditions can be seen in Figure 1. Vegetables exposed to plant litter of amaranth grown under hot temperature conditions showed the most significant inhibition in germination (Figure 1). Peppers were the most sensitive with 60 % germination inhibition. Lettuce was the least affected with only 22 % inhibition and affected more negatively by amaranth litter from plants cultivated at optimal temperatures than by that of plants grown at stress temperatures (Figure 1). It appeared as though germination of cucumber, pepper and tomato seeds were generally more sensitive to amaranth litter than lettuce. Steinsiek et al. (1982) reported a more severe allelopathic effect of an aqueous extract of common wheat (*Triticum aestivum* L) straw on the inhibition of germination and growth of selected weed species at 35 °C than at 25 °C or 30 °C. Einhellig & Echrich (1984) claimed that grain sorghum (*Sorghum bicolor* (L.) Moench.) and soybean (*Glycine max* (L.) Merr.) were more susceptible to ferulic acid when they were grown at higher temperatures. It was also observed that at 26/22 °C (day/night) temperature, the allelopathic inhibition of chlorogenic acid or tomatine was greater on the growth of an insect herbivore, *Manduca sexta* (Linnaeus, 1763) than at 26/14 °C (Stamp & Osier, 1997).

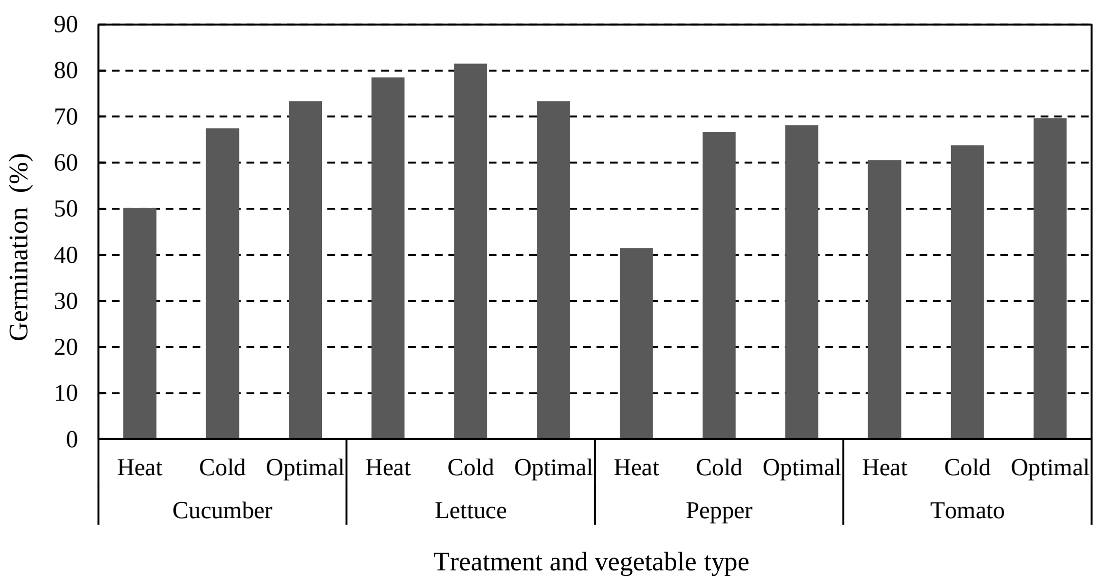

Figure 1: Response of the germination percentages of various vegetables to amaranth litter from plants grown at different temperatures. The data are expressed as average values. P < 0.01 ($LSD_{(T:0.05)}$ = 2.71). n = 96.

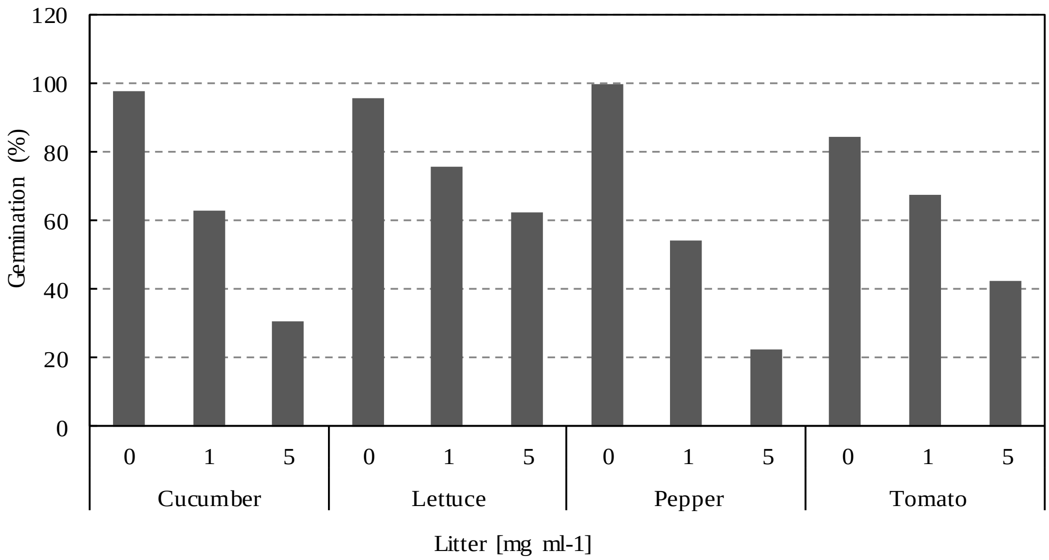

Figure 2: Influence of increasing litter concentration on the germination percentage of four vegetables ($LSD_{(T:0.05)}$ = 2.71). n = 96

Our results emphasise the importance of *A. cruentus* litter concentration on germination and growth of vegetables. The significant interaction between amaranth litter concentration and the effect on the four vegetable species is illustrated in Figure 2 and Table 1. Here it is clear that germination percentage and growth of all four vegetables decreased significantly with increased concentration of amaranth litter that was added to the growth medium, however, each vegetable species showed different levels of sensitivity. Peppers were the most sensitive to amaranth litter with the germination percentage falling from 100 % through 54 % to 22 % at the 0, 1 and 5 mg ml^{-1} litter concentrations respectively. Tomatoes were the least affected, with germination inhibition of only 17 % and 43 %, followed by 19 % and 33 % for lettuce and 36 and 66 % for cucumber at 1 and 5 mg ml^{-1}. From Table 1, an inhibition of 34 %, 76 % and 78 % in hypocotyl growth and 35 %, 68 % and 85 % in seminal root growth occurred in cucumber, pepper and tomato respectively at 1 mg ml^{-1}. An increase in concentration to 5 mg ml^{-1} resulted in growth inhibition of 65 % for cucumber and more than 90 % for pepper and tomato (Table 1).

Table 1: Effect of different concentrations of amaranth litter on the average hypocotyl and seminal root lengths of various vegetables

Leaf litter [mg ml^{-1}]	Hypocotyl length (mm)		
	Cucumber	Pepper	Tomato
0	33.15 ± 23.82	18.49 ± 13.99	39.56 ± 13.82
1	21.74 ± 24.62	4.39 ± 5.45	8.62 ± 9.93
5	11.87 ± 15.19	1.67 ± 2.64	2.05 ± 4.77
AVG	22.25	8.18	16.74
$LSD_{(T \leq 0.05)}$	11.06	4.44	4.67
Leaf litter [mg ml^{-1}]	Seminal root length (mm)		
	Cucumber	Pepper	Tomato
0	23.59 ± 10.32	13.38 ± 5.08	26.69 ± 6.67
1	15.36 ± 15.94	4.33 ± 5.76	4.08 ± 3.09
5	8.82 ± 10.58	1.23 ± 2.06	2.54 ± 2.89
AVG	15.92	6.31	33.31
$LSD_{(T \leq 0.05)}$	6.45	2.33	2.28

n = 13.

According to Qasem (2010), allelopathy plays a significant role in seed dormancy, seed germination and seedling emergence. The influence of allelochemicals on the balance of plant populations and species stability, microorganisms, natural enemies and insect populations, and the spread of pathogens is another important role of allelopathy (Qasem, 2010).

From the results it is clear that germination of all vegetables tested, was adversely affected by both concentrations of amaranth litter irrespective of the temperature treatment to which the plants were exposed (Figure 3). Analysis from our study therefore indicated that germination percentage was significantly affected by growth temperatures (T) ($P < 0.0001$) of the amaranth, litter concentration (C) ($P < 0.0001$), vegetable type (V) ($P < 0.0001$), the T × V interaction ($P = 0.0041$) and V × C interaction ($P < 0.0001$). The T × V × C ($P = 0.0540$) interaction showed no statistical differences.

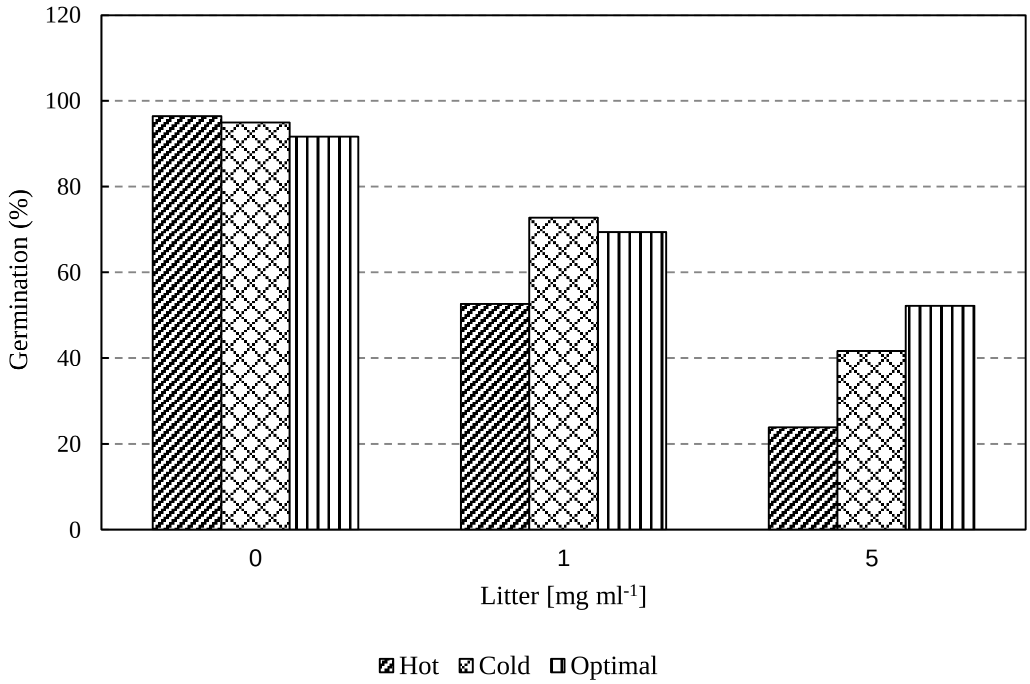

Figure 3: Two way interaction (ANOVA) between litter concentration and temperature on germination percentage of vegetables. $P < 0.01$ ($LSD_{(T:0.05)} = 2.73$). $n = 96$

Lettuce growth exhibited a two way interaction between concentration of plant litter and temperature treatment (Figure 4). Litter concentration and temperature treatments were highly significant in reducing hypocotyl ($LSD_{(T \leq 0.05)} = 3.43$) and seminal root ($LSD_{(T \leq 0.05)} = 3.41$) lengths respectively. From Figure 4 it is clear that the hot and optimal temperatures at both concentrations of litter, had a more severe effect on growth than the cool temperature treatment. Concentrations of 5 mg ml^{-1} for all the treatments inhibited hypocotyl and seminal root development almost completely.

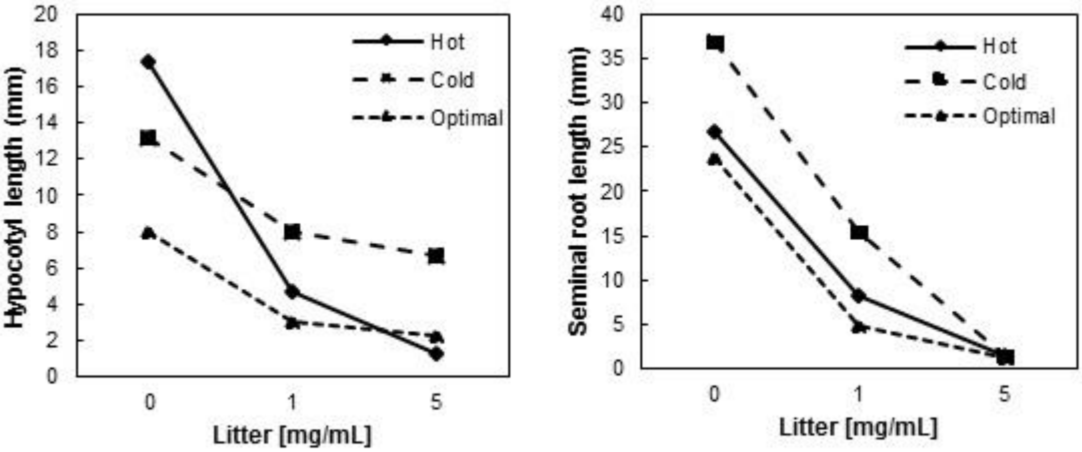

Figure 4: Results of two-way ANOVA of the effect of litter concentration and temperature and their interaction on hypocotyl ($LSD_{(T \leq 0.05)}$ = 3.43) and seminal root lengths ($LSD_{(T \leq 0.05)}$ = 3.41) of lettuce seedlings. *n* = 13.

It is demonstrated in this study that the environment for the cultivation of *A. cruentus* is important and that compounds responsible for allelopathy, are present even at optimal growth conditions. Furthermore, with increased concentrations of litter a decrease in germination and seedling development occurred. Therefore, it is possible that more leftover plant material in a field can have a more severe allelopathic effect on the following harvest. It was also clear that vegetables displayed diversity in reaction towards the temperature treatments and type of extract. In crop rotation systems it is important, because if germination is affected by compounds *of A. cruentus* produced under stress conditions, farmers can incur financial losses.

4 CONCLUSION

The *in vitro* bioassay results of the plant litter from *A. cruentus* indicated that the allelopathic compounds inhibiting germination and growth of vegetables are produced by amaranth irrespective of cultivation temperature, although the litter of plants grown under stressful conditions have a greater allelopathic effect than those at optimal environments. A difference in the response of vegetables and sensitivity to increasing concentration may have a negative impact in crop rotation systems. Results showed that it is vital to understand under which conditions amaranth was cultivated. Field trials will contribute to the confirmation of the phytotoxicity of the leaf litter and will be conducted in future studies. This will add to the understanding of allelopathic properties of *A. cruentus* in crop rotating systems.

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