

Full Length Research Paper

# Allelopathic potential of *Arctotis arctotooides* (L.f.) O. Hoffm aqueous extracts on the germination and seedling growth of some vegetables

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**Aqueous shoot and root extracts of *Arctotis arctotooides* were tested for their allelopathic properties on cabbage, carrot, tomato and spinach seed germination and seedling growth. The addition of the two extracts at 8 and 10 mg ml<sup>-1</sup> resulted in the highest germination inhibitions. The shoot extract at 10 mg ml<sup>-1</sup> inhibited cabbage, carrot, tomato and spinach seed germination by 79.1, 75.6, 82.0 and 46.0%, respectively while the root extracts at the same concentration had inhibitions of 66.0, 64.0, 64.7 and 42.1%, respectively on each of the vegetables. The decreases in the radicle growth of the four targets were highest in 10 mg ml<sup>-1</sup> of the shoot extract with cabbage, carrot, tomato and spinach having 95.3, 98.9, 92.9 and 87.6%, respectively. Considerable reductions also occurred in the plumule length of all the seedlings. The present study has demonstrated the inhibitory properties of the shoot and root aqueous extracts of *A. arctotooides* on the germination and seedling growth of the four vegetables.**

**Key words:** *Arctotis arctotooides*, allelopathy, germination, seedling growth, vegetable seeds.

## INTRODUCTION

Medicinal plants are becoming integral components of many subsistent farming systems in most developing nations of Africa and Asia due to the increasing awareness of human needs for wild indigenous plants as herbal remedies. Contrary to the acclaimed curative effects of several groups of plant's secondary metabolites, accumulation of these organic components in the soil negatively affect seed germination and seedling growth of other vegetations through a phenomenon called allelopathy (Omezzine et al., 2009; Umer et al., 2010). Allelopathy is an ecological and chemical interaction characterized by stimulatory and inhibitory effects among different plant families. Worldwide, the inhibitory properties of the extracts and residues of many herbal species co-habiting with desired crops on the same field have been a major source of concern (Hussain et al., 2007; Nazir et al., 2007).

One of such medicinal species commonly found in most disturbed habitats particularly on farms and research

fields around the University of Fort Hare, Eastern Cape Province of South Africa is the *genus Arctotis* of which *arctotooides* is a species (Pooley, 1998). *Arctotis arctotooides* (L.f.) O. Hoffm is a member of the family *Asteraceae* and an herbaceous perennial plant, native to the coastal district and summer rainfall areas of Southern Africa. The plant is locally known as *Ubushwa* in IsiXhosa and African daisy (English). At maturity, it grows up to 55 to 60 cm in height and the aerial part of the plant is usually covered with white hairy structures. Morphologically, these structures are believed to be special appendages through which plants' secondary metabolites are secreted (Cantrell et al., 2007). Oyedeki et al. (2005) reported the presence of crude compounds such as phenolics, sesquiterpene, monoterpene, coumarins, alkaloids, 1, 8-cineole and limonene in the essential oils of the two plant's parts of *A. arctotooides*. Qualitatively and quantitatively, the inhibitory effects of these phytochemicals on seed germination and healthy growth of susceptible crops and weed species have been well documented (Khan et al., 2011).

In many parts of the world, where allelopathy is viewed as a biological agent of weed control in agricultural production different methodological research have

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examined the allelopathic interactions that exist between several plant species and crop cultivars (Terzi, 2008; Khan et al., 2009). For example, Fischer (1986) and Einhellig and Leather (1988) indicated that monoterpenes, sesquiterpenes and alpha-pinenes from the essential oils of certain plants and crop cultivars inhibit seed germination and cause anatomical and physiological changes in the seedling growth of many targets. Mungole et al. (2010) confirmed the adverse effects of one or combined influence of phytotoxic chemicals in the extracts of medicinal plants on crops and weeds. Hassan and Ghareib (2009) found that a number of organic constituents released into the rhizosphere via mulching with straw and residue incorporation negatively affect the growth and yields of domesticated crops. On the contrary, in this part of Africa, where farmers combine wild indigenous plants with the existing farming operations, there is dearth of information on this topic.

The objective of the present study therefore, aimed to investigate the allelopathic effects of the shoot and root aqueous extracts of actively growing *A. arctotooides* plants on some widely grown vegetable (cabbage, carrot, spinach and tomato) seeds in Nkokonbe Municipality, Eastern Cape Province of South Africa.

## MATERIALS AND METHODS

Actively growing plants of *A. arctotooides* at the flowering stage were harvested from their naturally infested populations and on farms around the University of Fort Hare by uprooting in December, 2009. The plant was identified at the Department of Botany of the Institution and a voucher specimen deposited in the herbarium. The seeds of the test crops including cabbage (cv. Drumhead), carrot (cv. Kuroda), spinach (cv. Fordhook Giant) and tomato (cv. Heinz HT G08004) were obtained from Umutiza, a commercial seed centre located in Alice.

### Preparation of aqueous extracts

Aqueous extracts of the dried shoot and root parts of *A. arctotooides* were prepared by adopting the method described by Pandey et al. (1993) with little modification. The two plant materials were rinsed repeatedly in succession under running tap and distilled water to remove dust and soil particles before air-drying at room temperature for 24 h. Samples were further oven-dried at 60°C for 48 h (Pandey et al., 1993). The dry matter yields were ground in a hammer mill fitted with 1 mm sieve and stored separately in tightly sealed bottles at -20°C. 50 g of each powdery material was extracted in 1.5 L of distilled water and agitated on an orbital shaker for 12 h at room temperature. The filtrate was freeze dried at -50°C under the vacuum (RVT4104, USA) and reconstituted in distilled water to obtain the desired concentrations of 10, 8, 6, 4 and 2 mg ml<sup>-1</sup> following the procedures of Ashrafi et al. (2008) as a model. The pH value of each extract solution was determined immediately before incubation using the method described by Okalebo et al. (2002).

### Viability test

The viabilities of cabbage, carrot, tomato and spinach seeds were

assessed by adopting Kambizi et al. (2006) procedures. The objective of this protocol was to ascertain the quality of the test seeds based on their germination percentages. This invariably provides the basis for evaluating the allelopathic properties of the two aqueous extracts of *A. arctotooides*. 25 seeds of the individual vegetable randomly selected and placed into 9 cm Petri dishes were imbibed in 30 ml of distilled water for 24 h at room temperature. The fully expanded seeds were dissected into half and each half was further treated with 0.01% of 2,3,5-tetraphenyl tetrazolium chloride (TTC) and kept in the dark for 16 h at room temperature (Kambizi et al., 2006). At the end of the period, the set up was rinsed in several changes of distilled water and viewed under the light microscope for reddish colouration of the cotyledon.

### Seed germination and seedling growth studies

For seed germination studies, the effects of the dried shoot and root aqueous extracts of *A. arctotooides* at the five concentrations were evaluated using the method of Ashrafi et al. (2008). 25 seeds of each vegetable were placed evenly in 9 cm Petri dishes lined with two Whatman filter papers and moistened at once with 6.5 ml of the respective extract concentrations. Distilled water served for the control. The dishes were sealed with parafilm and incubated at 27 ± 2°C in a (Scientific Series-O 2000) growth chamber without illumination for 14 days (Amoo et al., 2008).

### Statistical analysis

The experimental design was completely randomized with four replications. The initial seed germination count was carried out after 72 h of incubation while the final germination and percentage of inhibition were evaluated after 14 days, at the end of the experiments. The declines in the radicle and plumule growth of the treated vegetable seedlings relative to the control were also assessed on the same day using the formula (Jefferson and Pennacchio, 2003). The analysis of variance (ANOVA) was performed on the data generated using Statistical Analysis System programme (SAS, 1999, Users guide: SAS Institute NC.) and treatment means separated by Duncan's Multiple Range Test at a 5% probability level.

## RESULTS AND DISCUSSION

The results of the tetrazolium test showed that the viabilities of cabbage, carrot, tomato and spinach seeds were 96, 92, 100 and 71%, respectively. The higher percentage viability recorded for cabbage, carrot and tomato seeds support (Leist and Krämer, 2003) described viability test as estimate of seed quality and planting values. The slightly lower viability (71%) observed in the spinach seeds might be attributed to other factors such as seed maturity, extraction methods, storage and possibly the nature of the seed coat (Bewley, 1997; Nerson, 2002). In all cases, the results indicated that the four vegetable seeds used in this study were of high quality thus providing a good platform for assessing the allelopathic potentials of the dried shoot and root aqueous extract of *A. arctotooides*.

The pH values of the two extracts ranged between 5.94 to 6.70 and 6.75 to 6.91, respectively but those obtained from the shoot extract were however not consistent with

**Table 1.** Effects of the different concentrations of the shoot and root aqueous extracts of *A. arctotooides* on the germination of some vegetable seeds.

| Extract concentration<br>(mg ml <sup>-1</sup> ) | Shoot extract      |                    |                    |                    | Root extract       |                    |                    |                    |
|---|--------------------|--------------------|--------------------|--------------------|--------------------|--------------------|--------------------|--------------------|
|   | Cabbage            | Carrot             | Tomato             | Spinach            | Cabbage            | Carrot             | Tomato             | Spinach            |
| 2   | 17.00 <sup>e</sup> | 23.00 <sup>e</sup> | 15.00 <sup>d</sup> | 13.00 <sup>d</sup> | 9.00 <sup>e</sup>  | 14.00 <sup>d</sup> | 9.00 <sup>d</sup>  | 7.00 <sup>c</sup>  |
| 4   | 33.00 <sup>d</sup> | 37.00 <sup>d</sup> | 20.00 <sup>d</sup> | 17.00 <sup>d</sup> | 28.00 <sup>d</sup> | 15.00 <sup>d</sup> | 9.00 <sup>d</sup>  | 8.00 <sup>c</sup>  |
| 6   | 52.00 <sup>c</sup> | 41.00 <sup>c</sup> | 33.00 <sup>c</sup> | 28.00 <sup>c</sup> | 42.00 <sup>c</sup> | 33.00 <sup>c</sup> | 27.50 <sup>c</sup> | 13.00 <sup>c</sup> |
| 8   | 67.00 <sup>b</sup> | 63.40 <sup>b</sup> | 64.80 <sup>b</sup> | 39.00 <sup>b</sup> | 61.00 <sup>b</sup> | 58.00 <sup>b</sup> | 48.00 <sup>b</sup> | 28.00 <sup>b</sup> |
| 10  | 79.20 <sup>a</sup> | 75.00 <sup>a</sup> | 82.00 <sup>a</sup> | 46.00 <sup>a</sup> | 66.00 <sup>a</sup> | 64.00 <sup>a</sup> | 64.70 <sup>a</sup> | 42.10 <sup>a</sup> |

\*Mean values followed by different letters in the superscript along the column differ significantly ( $P < 0.05$ ).

the different concentrations. Previous studies have reported that changes in extract concentrations are not likely to cause significant changes in the pH values but the responses of the test targets could be dramatic (Al-saadawi et al., 1986; Hartung et al., 1990). Therefore, the differences in the germination inhibitions and retardations in the seedling growth of the four vegetables under the various extract concentrations could be ascribed to the inhibitory effects of the applied aqueous treatments. The ANOVA results on the allelopathic potentials of the two *A. arctotooides* plant materials showed that the potency of the shoot and root aqueous extracts on the germination and seedling growth of cabbage, carrot, tomato and spinach was concentration-dependent.

The effects of the shoot extracts at the varying concentrations significantly inhibited the germination of all the vegetable seeds. The extract at 10 mg ml<sup>-1</sup> reduced the germination of cabbage, carrot, tomato and spinach seeds by 79.1, 75.6, 82.0 and 46.0%, respectively. However, there were no significant treatment effects in tomato and spinach seeds incubated with 2 and 4 mg ml<sup>-1</sup> of the shoot extract (Table 1). The impact of the root extracts on seed germination inhibitions was consistent with extract concentrations but the percentages were statistically lower than those treated with the shoot extract (Table 1). Generally, the responses of the four vegetable seeds with the exception of cabbage to the addition of the root extracts at 2 and 4 mg ml<sup>-1</sup> were not significantly different. Spinach seeds incubated with 6 mg ml<sup>-1</sup> of the extract also followed similar trend with those treated with the two lowest concentrations.

An et al. (1997) described seed germination as a valuable index in allelopathic studies. Exposure of seeds to one or combined influence of organic compounds such as phenolic acids, alkaloids and volatile terpenes in the extracts or essential oils of different medicinal species often results in negative physiological effects on the germination and seedling growth (Mungole et al., 2010). During germination, the action of gibberellic acid which induces the production of  $\alpha$ -amylase (an enzyme responsible for degradation of reserved carbohydrate to soluble sugars) is disrupted by the phytotoxic chemicals [Aghajanzadeh et al., 2007; University of Mazandaran, Journal of Basic Science (In Press)]. Einhellig (1995) also

reported that alteration in the enzymatic activity of seeds affect the mobility of stored compounds thus leading to complete or higher percentage of germination inhibitions. Therefore, the observed differences in the number of germinated seeds between the control and the treatments might be attributed to the presence of allelopathic compounds including phenolic (caffeic, vanilic and ferulic) acids and sesquiterpenes earlier isolated from the essential oils of the two *A. arctotooides* plant's parts.

When compared with the control, the effects of the shoot and root aqueous extracts at the varying concentrations significantly suppressed the radicle and plumule length of all the vegetable seedlings (Tables 2 and 3). The reductions in the radicle elongations of cabbage, carrot, tomato and spinach were highest in 10 mg ml<sup>-1</sup> of the shoot extract with each of the vegetables having 95.3, 98.9, 92.9 and 87.6% inhibition, respectively. Generally, the inhibition percentages resulting from the effects of the treatments on the plumule length of the four targets also followed similar growth dynamics with those of the radicles but they were much lower than in the radicle growths. For example, the reduction values of 48.2, 51.8, 46.9 and 46.3% recorded for cabbage, carrot, tomato and spinach plumule elongations as influenced by 8 mg ml<sup>-1</sup> of the shoot extract were not comparable to 67.8, 75.2, 63.6 and 60.5%, respectively observed in the radicles of the same set of species under 6 mg ml<sup>-1</sup> of the same extract. Apart from reductions in the two growth variables, the impact of the shoot and root extracts at 6, 8 and 10 mg ml<sup>-1</sup> also caused massive twisting of the radicles of all the treated seedlings with the exception of spinach.

In addition to brownish colouration of the radicle tips, the plumules of the affected targets also appeared weaker and feeble to touch. The effects of the root extract at 10 mg ml<sup>-1</sup> inhibited the radicle length of cabbage, carrot, tomato and spinach by 83.8, 86.5, 82.2 and 66.5%, respectively (Table 3). The reductions in the radicle growths of cabbage and carrot incubated with 6 and 8 mg ml<sup>-1</sup> of the extract also differed considerably whereas those of tomato and spinach incubated with 2 mg ml<sup>-1</sup> of the same extract were not significantly different from the control. Generally, the declines in the plumule

**Table 2.** Effects of the different concentrations of the shoot aqueous extracts of *A. arctotoides* on the radicle and plumule growth of some vegetables.

| Extract concentration<br>(mg ml <sup>-1</sup> ) | Seedling length (mm) |                    |                    |                    |                    |                    |                    |                    |
|---|----------------------|--------------------|--------------------|--------------------|--------------------|--------------------|--------------------|--------------------|
|   | Cabbage              |                    | Carrot             |                    | Tomato             |                    | Spinach            |                    |
|   | Radicle              | Plumule            | Radicle            | Plumule            | Radicle            | Plumule            | Radicle            | Plumule            |
| Control   | 45.91 <sup>a</sup>   | 57.36 <sup>a</sup> | 42.19 <sup>a</sup> | 55.34 <sup>a</sup> | 53.25 <sup>a</sup> | 83.05 <sup>a</sup> | 56.45 <sup>a</sup> | 66.23 <sup>a</sup> |
| 2   | 38.53 <sup>b</sup>   | 51.75 <sup>b</sup> | 30.18 <sup>b</sup> | 48.86 <sup>b</sup> | 38.23 <sup>b</sup> | 78.64 <sup>b</sup> | 45.87 <sup>b</sup> | 58.55 <sup>b</sup> |
| 4   | 34.07 <sup>c</sup>   | 42.80 <sup>c</sup> | 19.78 <sup>c</sup> | 35.67 <sup>c</sup> | 32.10 <sup>c</sup> | 74.35 <sup>c</sup> | 42.75 <sup>c</sup> | 49.78 <sup>c</sup> |
| 6   | 14.76 <sup>d</sup>   | 30.39 <sup>d</sup> | 10.45 <sup>d</sup> | 19.29 <sup>d</sup> | 19.40 <sup>d</sup> | 63.77 <sup>d</sup> | 28.10 <sup>d</sup> | 42.83 <sup>d</sup> |
| 8   | 5.19 <sup>e</sup>    | 11.72 <sup>e</sup> | 1.80 <sup>e</sup>  | 7.63 <sup>e</sup>  | 9.74 <sup>e</sup>  | 48.28 <sup>e</sup> | 12.07 <sup>e</sup> | 18.63 <sup>e</sup> |
| 10  | 2.15 <sup>e</sup>    | 4.70 <sup>f</sup>  | 0.44 <sup>e</sup>  | 2.35 <sup>e</sup>  | 3.80 <sup>f</sup>  | 19.24 <sup>f</sup> | 7.26 <sup>f</sup>  | 13.75 <sup>f</sup> |

\*Mean values followed by different letters in the superscript along the column differ significantly (P < 0.05).

**Table 3.** Effects of the different concentrations of the root aqueous extracts of *A. arctotoides* on the radicle and plumule growth of some vegetables.

| Extract concentration<br>(mg ml <sup>-1</sup> ) | Seedling length (mm) |                    |                    |                    |                    |                    |                    |                    |
|---|----------------------|--------------------|--------------------|--------------------|--------------------|--------------------|--------------------|--------------------|
|   | Cabbage              |                    | Carrot             |                    | Tomato             |                    | Spinach            |                    |
|   | Radicle              | Plumule            | Radicle            | Plumule            | Radicle            | Plumule            | Radicle            | Plumule            |
| Control   | 45.91 <sup>a</sup>   | 57.36 <sup>a</sup> | 42.19 <sup>a</sup> | 55.34 <sup>a</sup> | 53.25 <sup>a</sup> | 83.05 <sup>a</sup> | 56.45 <sup>a</sup> | 66.23 <sup>a</sup> |
| 2   | 40.76 <sup>b</sup>   | 55.98 <sup>a</sup> | 36.68 <sup>b</sup> | 50.19 <sup>b</sup> | 50.77 <sup>a</sup> | 81.60 <sup>a</sup> | 54.20 <sup>a</sup> | 63.54 <sup>b</sup> |
| 4   | 38.29 <sup>c</sup>   | 51.72 <sup>b</sup> | 24.90 <sup>c</sup> | 48.50 <sup>b</sup> | 44.18 <sup>b</sup> | 78.19 <sup>b</sup> | 50.75 <sup>b</sup> | 58.85 <sup>c</sup> |
| 6   | 24.98 <sup>d</sup>   | 47.25 <sup>c</sup> | 17.36 <sup>d</sup> | 43.32 <sup>c</sup> | 38.95 <sup>c</sup> | 73.84 <sup>c</sup> | 37.72 <sup>c</sup> | 50.97 <sup>d</sup> |
| 8   | 16.74 <sup>e</sup>   | 32.93 <sup>d</sup> | 9.44 <sup>e</sup>  | 36.84 <sup>d</sup> | 26.14 <sup>d</sup> | 54.85 <sup>d</sup> | 29.64 <sup>d</sup> | 42.06 <sup>e</sup> |
| 10  | 7.45 <sup>f</sup>    | 18.4 <sup>e</sup>  | 5.68 <sup>f</sup>  | 19.03 <sup>e</sup> | 9.48 <sup>e</sup>  | 39.32 <sup>e</sup> | 18.93 <sup>e</sup> | 35.94 <sup>f</sup> |

\*Mean values followed by different letters in the superscript along the column differ significantly (P < 0.05).

length were less severe in comparison with radicle elongations. For instance, the inhibition percentages (67.8, 65.6, 65.9 and 45.7%) in the plumule length of cabbage, carrot, tomato and spinach due to 10 mg ml<sup>-1</sup> of the root extract were not comparable to 83.8, 86.5, 82.2 and 66.5% reductions recorded for their respective radicles under similar treatments. These observations conform to Hegazy and Fadl-Allah (1995) who reported that the impact of allelopathic plant extracts on seedling growth is more important than seed germination.

Similarly, Ashrafi et al. (2008) stated that an extract concentration of as low as 1/25th was sufficient to completely reduce the radicle length. Thus, the drastic reductions of 95.3, 98.9, 92.9 and 87.6% as well as 83.8, 86.5, 82.2 and 66.5% recorded for cabbage, carrot, tomato and spinach incubated with *A. arctotoides* shoot and root extracts at 10 mg ml<sup>-1</sup> support earlier findings of Ashrafi et al. (2008). The wide variations between the inhibitory effects of the two extracts on the radicle growth of the affected seedlings relative to their plumule elongations might be attributed to close proximity of this organ to the phytotoxic substances in the extract (Romero-Romero et al., 2005; Hussain et al., 2007). El-Khatib (2000) also indicated that the susceptibility of

young targets to allelopathic plant extracts or specific allelochemicals increases with formation of some morphological abnormalities. Interestingly, the observed twisting and brownish colouration of cabbage, carrot and tomato radicles support the aforementioned statement.

Globally, species sensitivity and extract concentration are two important factors considered for accuracy in allelopathic research (Calabrese and Blain, 2005; Daizy et al., 2006). In the present study, the seeds of four crops were selected because of their popularity among the local farmers and the most widely cultivated vegetables in areas predominantly dominated by *A. arctotoides* plants. The production of several classes of allelochemicals famous for stimulatory or inhibitory effects on susceptible targets has been associated with the concentrations of such organic components in the tissues and organs of different the different plant species (Khan et al., 2008). The strong inhibitory potentials exhibited by the dried *A. arctotoides* aqueous shoot extract on seed germination and seedling growth of the four vegetables might be ascribed to higher percentages of phytochemicals earlier reported from this part (Oyedeeji et al., 2005).

Although, the pattern of seed germination inhibitions as influenced by the addition of the two highest

concentrations of the shoot and root extracts was constant with seed viability percentages, conversely, the observed variations in the responses of the individual vegetable at the other concentrations could be attributed to species sensitivity as suggested by An et al. (1997). Furthermore, the percentages of seed germination inhibitions due to the applied aqueous treatments on each of the four species were in the order of cabbage > carrot > tomato > spinach. The impact of the two extracts on the radicle elongations of the affected vegetables also followed the trend of carrot > cabbage > tomato > spinach even though there were no statistically significant differences between the radicle growth of cabbage and carrot incubated with the shoot extract at 8 and 10 mg ml<sup>-1</sup>.

## Conclusion

The results of the present study has demonstrated the inhibitory effects of the dried aqueous shoot and root extracts of *A. arctotoides* on all the parameters evaluated under laboratory conditions. Among the four vegetables, the seed germination and seedling growth of spinach were more tolerant to the applied treatments than those of the other three vegetables. Carrot radicles were however, the most sensitive. Thus, these results have shown that seed germination was not the best measurement of allelopathic phytotoxicity. Therefore, an understanding of the allelopathic interactions of the extracts or residues of medicinal plants and crops growing together on the same field is an important research base with economic implications. Further investigations on the allelopathic effect of the dried shoot residue of *A. arctotoides* on the growth and yields parameters of other crops might be an added advantage.

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