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Effect of Aqueous Extract of some Windbreak Tree Leaves on Seed Germination and Seedling Growth of Squash

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Abstract: The effects of aqueous leaves extract of three types of windbreak trees, Acacia, Casuarina, and Eucalyptus, were tested at 0, 25, 50, 75, and 100 g.L^{-1} for their effect on squash (*Cucurbita pepo* L.) seed germination and seedling growth. A group of squash seeds was sown in pots and irrigated with aqueous extract concentrations until the germination stage, while another group of seeds was maintained for three weeks under the same treatments until the seedling stage. Treating seeds with aqueous extract of Acacia resulted in a significant decrease in the percentage of germination (GP) and the germination rate (GR) of the seeds. The aqueous extract of Eucalyptus also led to a slight decrease in the GR, while no effect was observed when the aqueous extract of Casuarina was used. Treatment of seedling, on the other hand, with an aqueous extract of all windbreak types decreased all of the seedling growth parameters, fresh and dry weight (FW and DW), water content (WC), length of petioles (LP), number of buds (NB) and number of leaves (NL). The three windbreak trees induced an allelopathic effect on squash seedling growth in particular, more than the squash seed germination, but at different degrees. Acacia extract resulted in the highest effect, while Eucalyptus and Casurina resulted in intermediate and low inhibitory effects respectively. In all three windbreaks, the inhibitory effect of the extract on the seed germination and seedling growth increased as the extract concentration increased from 0 to 100 g.L^{-1}.

Keywords: Allelopathic; Windbreak Trees; *Cucurbita pepo* L.; Aqueous Extract.

INTRODUCTION

Competition between plants for moisture, nutrients, light by direct means or through an inhibitory effect on the growth of the other plants is a common phenomenon in nature(Tanveer et al., 2010). The inhibitory effect of one plant over another plant's growth depended on the ability of one of the plants to release allelopathic chemicals that inhibit the growth of the other plants (Abu-Romman, 2016; Kluthe et al., 2018). These allelopathic compounds can be synthesized by and were found in all parts of the plant (roots, stem, rhizomes, leaves, fruits, and seeds (Vijayan, 2015), but the leaves have the highest contents of the allelopathic compounds (Kumari et al., 2016). The allelopathic compounds come in contact with the other competing plants by leaching from decomposed plant residual, roots perfusion, volatilization, plus some other process (Sikolia & Ayuma, 2018).

The effect on the growth of the other competing plants is achieved by impending its ability to absorb water, nutrient, and photosynthesis (Othman et al., 2018). Not only have more than 100 000 allelopathic compounds been identified including phenolic acid, hydroxamic, alkaloids, quinines, and others (Othman et al., 2018), but also, each could affect the growth of the competing plant by different mechanisms (Kluthe et al., 2018).

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Aqueous extracts of Casuarina trees reduced the germination rate and decreased the seedling height of pumpkin and eggplant plants (John et al., 2010) and germination rate, height, and weight of shoots and roots of wheat, maize, and sorghum seedlings (Patil & Hunshal, 2010).

The allelopathic effect of the aqueous extract of Casuarina was more potent and its effect increased as the aqueous concentration increased. (Dejam et al., 2014) found that treatment of eggplant seeds with aqueous extracts of Eucalyptus trees decreased the percentage and rate of germination, plant height, and plant fresh and dry weight, and the severity of the effect increased as the concentration of the aqueous extract increased. (Espinosa-Garcia et al., 2008) also found that treatment of melon and squash seeds with aqueous extract of Eucalyptus decreased the germination rate of both melon and squash and the length of the epicotyl, radicle, and plumule of the watermelon seedlings. (Hegab et al., 2016) reported that the aqueous extract of Eucalyptus reduced the water content of corn plants. (Alshareef & Alaib, 2018) indicated that treatment of cucumber seeds with the aqueous extract of Acacia trees reduced the percentage of germination, length of roots, and the fresh and dry weight of the shoots of the cucumber seedlings. The reduction in these parameters was directly proportional to the concentration of the applied aqueous extract. Aqueous extracts of Acacia were also found to reduce fresh and dry weight, plant height, water, chlorophyll, nitrogen, and phosphorous content of peas (Al-Wakeel et al., 2007).

(Hussain et al., 2020)reported that the adverse effect of Acacia aqueous extract on the leaf protein of lettuce could be due to the toxicity of the polyphenols of Acacia. This study aims to determine the extent of the allelopathy effect of the leaves of Casuarina, Eucalyptus, and Acacia trees on the seed germination and seedling growth of squash.

MATERIALS AND METHODS

Plant material, growth conditions, and treatments*:* The experiment was carried out by cultivating the commercial variety "Alexandria F1" of the squash plant (*Cucurbita pepo* L.) in the research station of the Faculty of Agriculture - University of Benghazi – Libya, during the summer seasons of 2019 and 2020. Seeds were sowen in silty clay soil mixed with sand and compost (1:1:1 vol.) in 3-liter pots. Soil composition was 1.27% organic matter, 20% calcium carbonate, pH 7.56, EC 0.98 dSm^{-1}, available P 10.4 ppm, and total N 0.11%.

Squash seeds were sowen in the germination substrate and irrigated by aqueous extract of the windbreak tree leaves, Casuarina (*Casuarina equisetifolia* L.), Eucalyptus (*Eucalyptus globulus* Labill.), and Acacia (*Acacia neriifolia* A.Cunn. ex Benth.) at different concentrations of aqueous extract. The aqueous extract concentrations were prepared by first grounding the dry leaves, then soaking certain weights in fresh water according to the required concentrations (0, 25, 50, 75, and 100 $g.L^{-1}$) for 24 hours at room temperature, and second, the extract was filtered and used immediately under field conditions for irrigation.

Throughout the experiment, the plants were fertilized by mixing modified Hoagland Solution as a complete nutrient solution (NS) with irrigation water. The full NS contains (in $mmol.L^{-1}$) 5 KNO_3, 5 $Ca(NO_3)_2 \cdot 4H_2O$, 2 $MgSO_4 \cdot 7H_2O$, 1 KH_2PO_4, 0.02 $FeSO_4 \cdot 7\ H_2O$; 0.02 Na_2- EDTA; 2 H_2O; 0.045 H_3BO_3; 0.01 $MnCl_2 \cdot 4\ H_2O$, and (in μmol/L) 0.8 $ZnSO_4 \cdot 7\ H_2O$, 0.4 $Na_2MoO_4 \cdot 2\ H_2O$, and 0.3 $CuSO_4 \cdot 5\ H_2O$. Seedlings were grown under a plastic cover for protection from rain at 14h photoperiod. Photosynthetic active radiation reached a daytime peak value of 1200 $\mu mol.m^{-2}.s^{-1}$, and the temperature and relative humidity were 29 and 18°C and 39 and 73% during the day and night periods respectively.

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Irrigation was scheduled according to seedling requirements and the substrate water holding capacity.

Germination stage measurements*:* Germination percentage (GP) was calculated by the following equation:

$$GP = \frac{\text{No.of seeds germinated}}{\text{total seeds}} \times 100$$

Germination rate (GR) was calculated according to the following formula of Bartlett (1937):

$$GR = \frac{a + (a + b) + (a + b + c) + (a + b + c + m)}{n(a + b + c + m)}$$

Where a, b, c are the number of seedlings in the first, second, and third count, m is the number of seedlings in the final count, n is the number of counts.

Vegetative stage measurements*:* Seedlings were cut off after three weeks from seed germination, fresh weight of seedlings (FW) were measured, then plants were dried for three days in an oven at 65 °C (until there was no decrease in weight) for determination of dry weight (DW) and percentage of water content (WC). The number of buds (NB) and leaves (NL) of the seedling were counted, and the length of petioles (LP) was measured.

Experimental design and statistical analysis*:* The data represent the mean of two independent experiments that were conducted for two seasons. The experiments layout included four replicates for each treatment (each replicate consist of ten seeds in one pot for the germination stage and four seedlings in four pots for vegetative stage measurements). Factorial experimental 3×5 in a completely randomized design was used with the treatments of windbreak tree types and aqueous extract concentrations. Data were subjected to analysis of variance using a two-way ANOVA, and means were compared by Duncan's Multiple Range test at probability 0.05 using the SAS GLM procedure (SAS Institute, Cary, NC).

RESULTS

The results (Figure 1 and 2) showed highly significant differences among the treatments for germination measurements of squash seeds. Aqueous extract of Acacia tree leaves decreased both GP and GR significantly compared to control and aqueous extract of the other windbreak trees. The value of the GP was not affected by the low concentration of aqueous extract of the Acacia species (25 g/L), while the value of the GR decreased to less than half compared to the control treatment and aqueous extract of the other windbreak trees. The treatment with a 50 g/L concentration of aqueous extract of Acacia reduced the GP to about half, and the severity of the effect increased as the aqueous concentration increased. The value of the GR decreased to lower levels as the aqueous extract concentration of Acacia leaves increased. The GP was not affected by the treatment with different concentrations of the aqueous extract of Casuarina and Eucalyptus leaves while the value of GR decreased significantly with all concentrations of the aqueous extract of the Eucalyptus leaves.

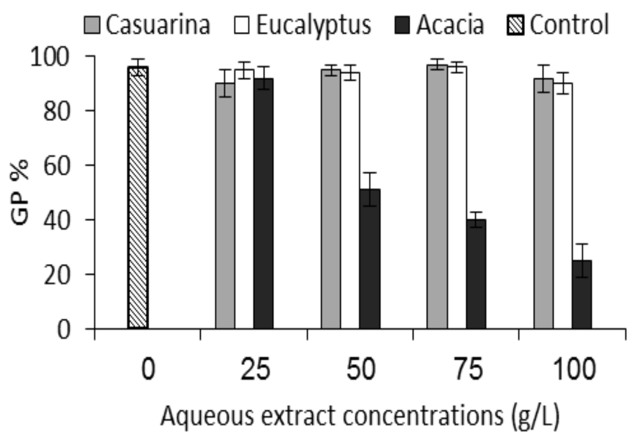

Figure 1. Effect of types and concentrations of aqueous extract of windbreak trees on germination percentage (GP) of squash seeds

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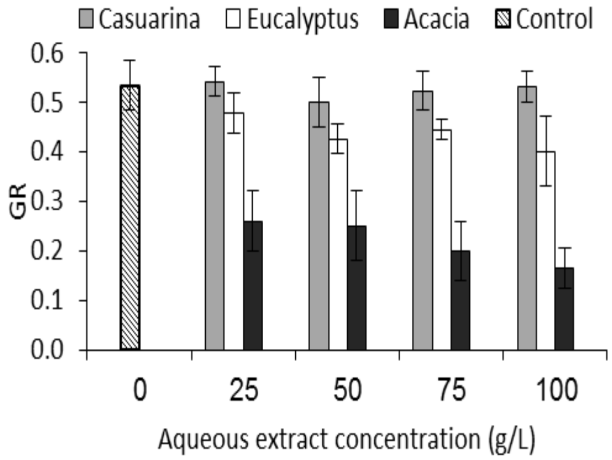

Figure 2. Effect of types and concentrations of aqueous extract of windbreak trees on germination rate (GR) of squash seeds

The results presented in Table 1. show a decrease in the growth of squash seedlings treated with different concentrations of aqueous extract of leaves from all three windbreak trees. The FW and DW of squash seedlings treated with aqueous extract of Eucalyptus and Acacia leaves decreased significantly compared to control. In addition, the decrease in FW and DW was inversely proportional to the increase in the concentration of the aqueous extract of all windbreak types. The higher the aqueous concentration, the lighter the FW and DW. On the other hand, at any of the different used concentrations, treatment with aqueous extract of acacia leaves resulted in significantly lower NB, NL, LP, and WC values than the control and the aqueous leaves extract of the other windbreak trees. In contrast, at any concentration, the effect of aqueous extract of Casuarina leaves did not show at any of the tested concentrations any significant effect on squash seedling growth.

Table 1. Effect of types and concentrations of aqueous extract of windbreak trees on fresh weight of plants (FW), dry weight of plants (DW), number of leaves (NL), number of buds (NB), length of petioles (LP) and water content, (WC) of squash seedlings.

Treatments		Measurements					
Tree Type	Extra. Conc. g/L	FW g/p.	DW g/p.	NB bud	NL Leaf	LP Cm	WC %
Con.	0	7.40^{a}	0.60^{a}	5.50^{a}	5.00^{a}	8.70^{a}	91.9^{a}
Cas.	25	7.68^{a}	0.68^{a}	5.50^{a}	5.00^{a}	8.25^{ab}	91.2^{a}
	50	7.18^{a}	0.58^{a}	5.33^{a}	5.00^{a}	8.08^{ab}	92.8^{a}
	75	5.88^{b}	0.45^{b}	5.50^{a}	4.25^{ab}	7.88^{ab}	92.0^{a}
	100	5.63^{b}	0.40^{b}	5.25^{a}	4.33^{a}	7.60^{b}	92.6^{a}
Euc.	25	5.28^{b}	0.44^{b}	5.20^{a}	4.20^{ab}	7.40^{bc}	91.7^{a}
	50	4.94^{b}	0.38^{bc}	5.60^{a}	4.20^{ab}	6.58^{c}	92.3^{a}
	75	3.50^{c}	0.28^{c}	4.80^{ab}	3.80^{bc}	5.52^{d}	91.9^{a}
	100	2.98^{c}	0.25^{c}	4.00^{bc}	3.50^{bc}	4.98^{de}	91.4^{a}
Aca.	25	3.04^{c}	0.25^{c}	3.14^{c}	3.14^{c}	4.83^{de}	91.0^{a}
	50	2.84^{c}	0.24^{c}	2.60^{c}	3.00^{c}	4.10^{ef}	88.8^{b}
	75	1.70^{d}	0.20^{cd}	0.80^{d}	2.50^{cd}	3.40^{fg}	88.1^{bc}
	100	1.26^{d}	0.13^{d}	0.50^{d}	2.00^{d}	2.46^{g}	86.4^{c}

Con. (Control), Cas. (Casuarina), Euc. (Eucalyptus), Aca. (Acacia). Each value represents the mean values of two independent experiments, four replicates for each experiment. Means followed by the same letter in each column are not significantly different by Duncan's multiple range test at 5% level.

DISCUSSION

The aqueous extract of Acacia leaves induced a very high significant effect in all growth and development stages of squash seedlings than the other aqueous extract of the other windbreaks trees (Figure 1 and Table 1). On the contrary, the growth and the developmen-

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tal stages of squash seedlings were not affected by the aqueous extract of Casuarina. The results obtained by treating with Casuarina extract was not different from the results of control treatment. Although the current study's results using the squash plant indicated the absence of allelopathic effect of Casuarina windbreak trees on squash seedlings, others have reported an allelopathic effect when tested with other plants (Dejam et al., 2014; Patil & Hunshal, 2010). It can be said that the acacia was the only tree that had a very strong allelopathic effect on squash germination and seedling growth. Using different crops, similar findings of strong Acacia allelopathic effects were reported (Al-Wakeel et al., 2007; Alshareef & Alaib, 2018).

Being that Acacia aqueous extract was more inhibitory than the aqueous extract of Casuarina and Eucalyptus on squash seedling growth, it could be attributed to the difference in components of their aqueous extracts and to the mechanism by which these three windbreak trees induced their allelopathic effect in the competing neighboring plant species to compete with for nutrients and water. The difference could also be attributed to the nature of the squash seeds and seedling responses to the allelopathic factors produced by the different windbreak trees (John et al., 2010).

Increasing the inhibitory effect of Acacia extract on the squash germination and seedling growth as the aqueous concentration increased indicated that the amount of allelopathic compound of the extract increased if more leaves were used for aqueous extraction (Choudhari et al., 2019). The allelopathic effect of the aqueous extracts of Casuarina and Eucalyptus trees depended on the aqueous concentration. Only a higher concentration of the aqueous resulted in significant reduction in the growth of squash seedlings. On the contrary, the effect of Acacia aqueous extract was not limited to higher aqueous extract concentrations. At any concentration, Acacia aqueous extract resulted in a marked reduction in germination and growth of squash. The Acacia aqueous extract effect on squash was consistent with its reported effect on other vegetable crops (González et al., 1995). Windbreaks are usually used in many vegetable farms and the type of windbreak should be selected based on its allelopathic effect on the type of vegetable crops being grown. The results of the current study indicated that Acacia is not a proper windbreak for squash farms, and if used, it should be placed at a proper distance away from the squash area. The allelopathic effect of aqueous extract of Casuarina and Eucalyptus trees depended on the stage of squash growth. While Casuarina had no significant effect on both of germination percentage, germination rate, and seedling growth, Eucalyptus had no effect on germination percentage (Figure 1) but induced a significant effect on germination rate (Figure 2) and seedling growth (Table 1). Germinated seeds of squash may have a higher ability to tolerate the allelopathic effect more than the growing seedling of squash. John et al. (2010) mention that the increase in the inhibitory effect of the extract as the aqueous extract concentration increased could be attributed to an increase of allelopathic substance in the aqueous extract.

CONCLUSION

The allelopathic effect of the aqueous extracts of the leaves of Casuarina, Eucalyptus and Acacia trees on the squash germination and seedling growth depended on the growth stage and the aqueous extract concentration. At any concentration, Acacia aqueous extract resulted in a lower seed germination percentage and rate (GP and GR), and the inhibitory effect of aqueous extract increased as the concentration increased. On the contrary, the aqueous extract of the leaves of the Casuarina trees induced no effect on both parameters of seed germination. The effect of aqueous extract of Eucalyptus, on the other hand, was

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intermediate between the strong effect of Acacia and none effect of Casuarina. In general, the allelopathic effect of all trees increased as the aqueous concentration increased, specifically when applied during the squash seedling stage. Acacia aqueous extract remained effective as it was during seed germination and the effect of Eucalyptus and Casuarina become more pronounced than it was during seed germination.

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تأثير المستخلص المائي لأوراق بعض أشجار مصدات الرياح على إنبات البذور، ونمو شتلات الكوسة

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المستخلص: تم اختبار تأثير مستخلص الأوراق المائي لثلاثة أنواع من أشـ جار مصـ دات الريـ ـاح، الأكاسـ ـيا، الكازوريـ ـا، والأوكالبتوس عند تركيزات 0، 25، 50، 75 و100 جم / لتر على إنبات البذور، ونمو شتلات الكوسة (*Cucurbita pepo* L.). زرعت مجموعة من بذور الكوسة في أصص، وسقيت بتركيزات المستخلص المائي حتى مرحلة الإنبات، بينما استمرت مجموعة أخرى من البذور بالمعاملة نفسها حتى مرحلة الشتلات لمدة ثلاثة أسابيع. أدت معاملة البذور بالمسـ تخلص المـ ـائي لنبات الأكاسيا إلى انخفاض معنوي في نسبة الإنبات (GP)، ومعدل إنبات (GR) البذور. أدى المستخلص المائي لأوكـ ـالبتوس أيضًا إلى انخفاض طفيف في GR، بينما لم يلاحظ أي تأثير عند استخدام المستخلص المائي من الكازورينا. من ناحية أخرى، أدت معاملة الشتلات بالمستخلص المائي لجميع أنواع مصدات الرياح إلى تقليل جميع مقاييس نمو الشتلات، الوزن الطـ ـازج، والجاف (FW وDW)، المحتوى المائي (WC)، طول الأعناق (LP)، عدد البراعم (NB)، والأوراق (NL). تسببت أشجار مصدات الرياح الثلاثة في إحداث تأثير اليلوباثي على نمو الشتلات أكثر من إنبات بذور الكوسة، ولكن بدرجات مختلفـ ـة مـ ـن التأثير. نتج عن مستخلص الأكاسيا أعلى تأثير، بينما نتج عن والأوكالبتوس، والكازورينا تأثير تثبيطي متوسط، ومنخفض على التوالي. لجميع مصدات الرياح الثلاثة، زاد التأثير المثبط للمستخلص على إنبات البذور، ونمو الشتلات مـ ـع زيـ ـادة تركيـ ـز المستخلص من 0 إلى 100 جم / لتر.

الكلمات المفتاحية: الأليلوباثي ؛ أشجار مصدات الرياح *Cucurbita pepo* L. ؛ مستخلص مائي.

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