Annals of Arid Zone 27 (3&4) : 171-177, 1988

DORMANCY AND CHANGES IN PHENOLIC COMPOUNDS OF *ARTEMESIA SCOPARIA* SEEDS

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ABSTRACT

Poor germination of fresh seeds of *Artemesia scoparia* (30% in husked and 5% in unhusked seeds) was found to be due to the relatively high percentage of phenolic constituents of the seeds (9.75) and their bracts (6.50). After one year of dry storage, when the total phenolic compounds decreased to 1.45% in seeds and 3.95% in bracts, the germinatlon of both husked and unhusked seeds improved to 98% and 30%, respectively.

INTRODUCTION

Artemisia scoparia Waldstetkit (Compositae), is a widely scattered and useful range shrub in the arid regions of Iraq (Al-Charchafchi et al., 1986). Some aspects of seed germination of this species have been studied (Al-Ani et al., 1971, Al-Charchafchi et al., 1986), but nothing is known about the observed seed dormancy and the possible role of the bracts surrounding the seeds on inhibition of germination.

Al-Charchafchi et al. (1987) indicated that phenolic compounds might be involved in the germination inhibition of *Artemesia herba-alba*. The studies were, therefore, undertaken to find out the endogenous phenolic compounds of *A. scoparia*, the possible changes during the dry storage and their effects on seed germination.

MATERIAL AND METHODS

Husked (bracts removed) and unhusked fresh seeds of *A. scoparia* were kept in paper bags and stored at room temperature in December 1984. Germination tests were carried for two years in glass petri dishes (9 cm dia) lined with a layer of Whatman No. 3 filter paper, and moistened with 5 ml of distilled water or test solutions. Fifty seeds per dish were replicated four times for each treatment. All tests were done in an incubator (Precision Model 806) set at 20°C and under continuous illumination. Counts of germination were made every other day for a period of 12 days.

The phenolic constituents were studied for the husked seeds and bracts at 1, 6 and 12 months after harvest, following Al-Charchafchi et al. (1987). Gradient concentrations were prepared from certain amount of total phenolic constituents and tested for their inhibitory action on non-dormant husked seeds of *A. scoparia.*

The phenolic compounds were separated by silica thin layer chromatographic plates (20 x 20 cm, 0.2 mm and 0.5 mm thickness for preparative scale) developed with 15% acetic acid/water mixture. The spots were detected by ammonia vapours, ferric chloride solution, and ethanolic solution of potassium hydroxide.

For the identification of the separated phenolic compounds two dimensional paper chromatography using Whatman 3MM (Mabry et al., 1970) and developed by the descending technique, was carried out with tertiary butanol/acetic acid/water mixture (TBA, 3:1:1) as the first and water/acetic acid mixture (15:85) as the second solvent.

The UV-spectra were recorded with SP8-250 UV/Vis Pye-Unicam Spectrophoto meter in ethanol; λ_{max} for the isolated compound from the seeds were 320, 299, 280, 246 nm and λ_{max} for the isolated compound from the bracts were 399 and 246 nm.

Accurately weighed amounts of these purified constituents were separately dissolved in distilled water and gradient concentrations were prepared to test their inhibitory action on the husked seeds of *A. scoparia.*

RESULTS AND DISCUSSION

Effects of the bracts and seed age on germination

Fresh husked seeds exhibited low percentage of germination in winter (Fig. 1). However, in the following spring and summer, the germination capacity improved progressively to a maximum of 98% after one year. Similar results were reported for *A. herba-alba* by Al-Charchafchi and Jawad (1982).

In unhusked seeds of *A. scoparia* germination did not exceed 30% throughout the two year storage period, clearly indicating the inhibitory effects of bracts (Fig. 1). The possibility of mechanical resistance to germination is ruled out by the fact that the bracts are open at the top. Al-Charchafchi et al. (1986) showed that as the seeds become older (98% germination) respiration significantly increases. Thus certain changes in the seed to take place which result in their better germination.

Changes in total phenolic contents during the dry storage

The Rf values and colour reactions of the separated spots are given in Table 1. The Rf values of the major spot of one month old seeds detected by this procedure were 0.82 and 0.65 in TBA and 15% acetic acid, respectively. From the seeds, 227 mg (Rf = 0.85) of the major flavonoidal constituents was separated and 25 mg

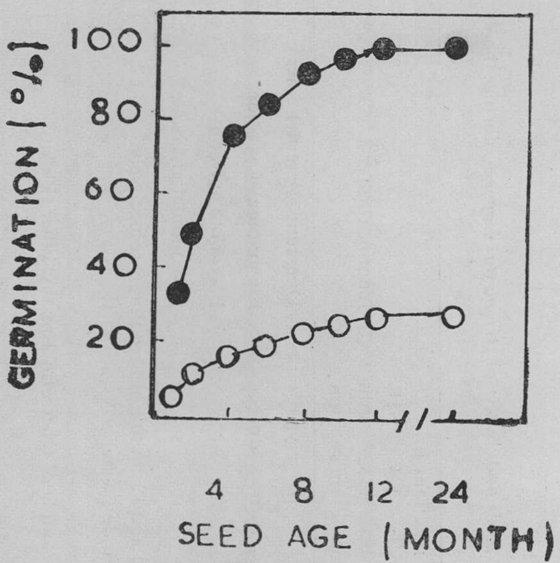

Figure 1. Effect of time lapse and the presence or absence of bracts arround the seeds on their germination. ● Husked, O Unhusked.

(Rf = 0.05) from the bracts. The total phenolic constituents decreased from the initial levels (one month after harvest) of 6.50% to 5.30% at 6 months and to 3.95% at 12 months after harvest. In the seeds, initial level of phenolic compounds one month after harvest (9.75%) dropped to 1.45% at 12 months after harvest.

Higher percentage of the total phenolic compounds left in the bracts even after one year explains the lower germination percentage of the unhusked seeds. Lower phenolase activity might be related to the lower activity of growth promoting substances in the dormant seeds.

Effects of the isolated phenolic compounds on germination and seedling development

The inhibitory effects of the phenolic compounds isolated from the seeds and the bracts at two stages (after harvest time) on germination of husked seeds and consequently on seedling development are shown in Figure 2. It is seen that germination sharply decreased as the applied concentration of the total phenolic compounds isolated from bracts increased (Fig. 2A). However, the total phenolic compounds isolated from 1-month old bracts decreased the germination significantly more (P=0.01) then phenolic compounds isolated from 12-month old bracts. In other words, total phenolic compounds isolated from the bracts of dormant seeds probably contained more inhibitory fractions. Comparable effects were observed with regards to seedling development (Figure 2B).

Table 1. : Identification and percentages of phenolic compounds of the seeds and bracts of *Artemesia scoparia* during the dry storage time.

Plant sample	[1]Spot No.	Rf in solvent*		**Colour reactions in				Identification
		I	II	Ethanolic KOH	$FeCl_3$ solution	UV 335 mμ	NH_3 solution	
Seeds	1(85,10)	0.85	0.90	+	+	p	yg	Quercetin-3-methyl ether
	2(15.90)	0.08	0.15	+	+	p	dp	Flavonoidal glycoside
Bracts	3(10,50)	0.53	0.70	—	+	p	p	Flavonoid aglycon
	4(90,50)	0.05	0 10	+	+	p	p	Flavonoidal glycoside

[1]Figures in parentheses denote percentage of phenolic compounds in 1-month old and 12-month old seedlings, respectively.

*Solvent I : 15% acetic acid in water; Solvent II : ethyl acetate : butanol : formamide : water :: 5 : 3 : 1 : 1.

**p = purple, dp = dark purple. y = yellow, g = green.

Note : the same Rf values and colour reactions were obtained with the authentic sample of Quercetin-3-m thyl ether (Fluka).

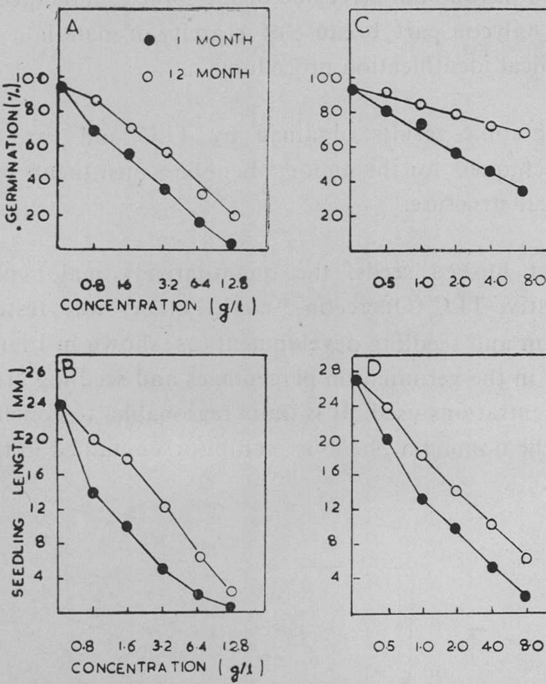

Figure 2. Effect of different concentrations of isolated endogenous phenolic compounds from the bracts (A,B) and husked seeds (C,D) on germination and seedling development of the non-dormant husked seeds of *A. scoparia*

Similar inhibitory effects of the phenolic compounds isolated from husked seeds on germinatlon were lower as compared to the inhibitory effect of such compounds isolated from bracts, at both the stages of isolation time (Fig. 2, C.D).

Identification of the phenolic compounds

The TLC of the phenolic fractions obtained from 1- and 12- month old seeds revealed the presence of similar compounds of flavonoidal type, but the quantitatively major compound detected in the 1- month old seeds was found as the minor compound in the 12 -month old seeds (similar Rf values in different solvent systems and the same colour reactions) and vice - versa (Table 1). This was possibly due to the enzymatic reactions during the dry storage period.

UV spectral analyses and colour reactions of the major phenolic compound of the 1- month old seeds confirmed it to be a 3-methoxy flavone derivative and possibly, Quercetin-3-methyl ether.

The TLC analyses and the colours observed under long UV showed that the other (flavonoidal) constituent of the seeds was different from that separated from the bracts (Table 1).

For the isolated flavonoidal glycoside of the bracts, no specific structure could be suggested for the aglycon part because of scarcity of material, not enough for the hydrolysis and chemical identification procedures.

All chromatographic results obtained by TLC and two dimensional paper chromatographic techiques for the major phenolic constituent were consistent with the suggested chemical structure.

In the dormant husked seeds, the quantitatively major phenolic compound separated by preparative TLC (Quercetin-3-methyl ether) was tested for its inhibitory action on germination and seedling development as shown in Figure 3. It shows that significant reduction in the germination percentages and seedling growth (P=0.01) was obtained by all concentrations used. It is quite reasonable to conclude that Quercetin-3-methyl ether was the dominant phenolic inhibitor contained in the dormant husked seeds of *A. scoparia*.

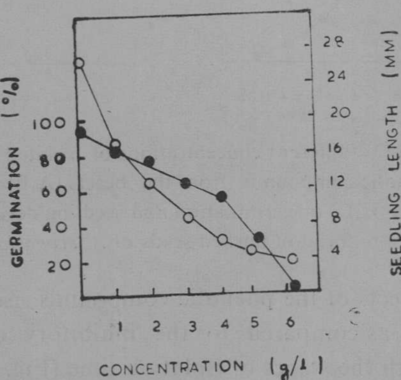

Figure 3. Effect of different concentration of isolated major phenolic compound from the dormant husked seeds on germination and seedling development of the non-dormant husked seeds.) ● Germination (%), O Seedling length (mm)

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