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SOME AUTECOLOGICAL STUDIES ON *AMARANTHUS VIRIDIS* L.

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ABSTRACT

Amaranthus viridis L. is a common wild vegetable and weed of cultivation. The studies indicated that seeds of Amarathus viridis L. germinated better at 25–30°C with or without any chemical/ mechanical treatment. The germination percentage decreased with increased sowing depth and water stress. Seeds did not germinate at all the applied levels of salinity. The plants grew better in diffused and partial light conditions. However, the growth was poor in water - stressed soils. The aqueous extracts from shoots, rain leachate, litter and root exudation significantly reduced the germination and seedling growth of pearl millet, wheat and corn. Shoot extract retarded the development of meristematic cells of test species. It was concluded that A. viridis germinates easily, grows better in diffused light with normal moisture condition and it possess allelopathic potential against crops.

INTRODUCTION

Amaranthus viridis L. (Family Amaranthaceae) is distributed in the warmer parts of the world including Pakistan. Of the 50 species of the genus *Amaranthus*, about a dozen are cosmopolitan weeds. *A. viridis* is a common annual weed of cultivation and waste land species from plains to 2200 m elevation throughout Pakistan (Ali, 1977). It serves as forage and as a wild leafy vegetable Autecological studies including germination, growth behaviour and allelopathy have been done on many weeds (Hussain *et al.*, 1987, 1989 & 1994; Inam *et al.*, 1989). However no such information exists on *A. viridis*. The present study, therefore, reports germination and growth performance under different ecological conditions and allelopathic behaviour of this weed.

MATERIALS AND METHODS

Germination studies under laboratory condition

Seeds were collected from Yar Hussain, Swabi, dried and stored at room temperature (25 – 30 C). Visually healthy seeds were tested for germination. The methods for sterilization of glassware, seeds, substrate etc and testing germination have been detailed elsewhere (Hussain *et al* , 1980 & 1993; Hussain and Nasrin, 1985; Hussain and Ilahi, 1988). Following experiments were performed

1. **Effect of different temperatures**

Seeds were incubated at a constant temperature of 15, 20, 30 and 35°C and alternating temperature between 15/20, 15/25, 15/30, 20/25, 20/30, 25/30 and 30/35 °C. The germination was recorded after every 48 hrs till 15 days.

2. **Effect of soaking in water**

Seeds were soaked in water at room temperature for 1, 2 and 4 hrs. Two other batches were immersed in hot (60°C) and boiling water (100°) separately for 1, 2 and 4 minutes. These treated along with non -- treated seeds were tried for germination at 25 and 30°C as before.

3. **Effect of scarification**

Seeds were chemically scarified with concentrated H_2SO_4, acetone, 0.25% sodium hyperchlorite for 1, 2 and 4 minutes.

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Mechanical scarification was accomplished by rubbing seeds in between sand papers till the testa ruptured at least from some parts. In another experiment, mechanically scarified seeds were treated with acetone or H_2SO_4, hyperchlorite as before. Treated seeds along with a control were tested for germination at 25 and 30°C.

4. Effect of pH

Seeds were grown separately at pH 2, 5. 7 and 10 at 25 and 30°C.

5. Effect of sowing depth

Chemically scarified seeds were sown at a depth of 4, 8 and 12 cm in sandy loam soil in glass container at 25°C. Observations continued for up to 30 days. Emergence of seedling from the soil was taken as index of germination.

6. Effect of different water levels

Seeds were grown in petridishes containing washed sand and with soil moisture maintained at field capacity (FC), ½ FC, ¼ FC, 1½ FC and 2 X FC at 25°C for up to 15 days. The dishes were kept at 25°C

For determining the field capacity, soil was saturated within a perforated container. It was allowed to stand for 72 hours and soil moisture determined by oven dry method following Hussain (1989). The soil moisture retained at this stage is the field capacity. Various levels such as ½ FC, ¼ FC, 1 ½ FC and 2 X FC were maintained by adding appropriate amount of water to soil in petri dish. Since it was short-term experiment therefore subsequent addition of water was not needed.

7. Effect of Salinity

Seeds were grown separately in 0.25, 0.50, 0.75 and 1 solutions of $MGSO_4$, $CaCl_2$, NaCl and KNO_3 at 25°C.

8. Growth performance under field condition

Seeds were sown in the 2nd week of February in pots containing loamy soil with 1% added organic matter. After one month of the emergence, seedlings were thinned to 5 healthy uniform plants in each pot. Hand weeding was done during the experiment. In each of the following experiments, there were five replicates each with 5 plants.

i. Effect of different light condition

Pots were kept under diffused light, partial light and full light condition in the garden. The soil moisture was maintained at field capacity level.

ii. Effect of different water levels

Field capacity of the soil in pots was determined following method of Mubarak *et al.* (1982). The pots were maintained at field capacity (FC), moderate stress (1/2 FC), severely stressed (1/4 FC) and moderately moist (1½ FC) and flooded (2 X FC).

Pots were weighed twice a week and any loss of water was compensated. The experiments lasted for 2 months. Height of plants, No of branches, leaves, length of inflorescence fresh and dry weight of leaves and roots were determined. After harvest plants were dried at 65°C for 72 hours. Chlorophyll contents of leaves were determined spectrosphotometrically following methods of Harborne (1963).

Allelopathic potential

Mature plants were collected from the Peshawar University campus and dried at room temperature (25°C). Sterilization of glassware, preparation, storage of extracts and bioassays have been previously described (Hussain *et al.*, 1987, 1989 & 1994; Inam *et al.*, 1989).

a. Effect of aqueous extract

Five gram dried or fresh crushed shoots were soaked in distilled water for 24 hrs and filtered. Extract was used against *Setaria italica*, *Pennisetum americanum*, *Zea mays* and *Triticum aestivum* in standard filter bioassay following Hussain *et al.* (1987, 1989 & 1994). There were 5 replicates, each with 10 seeds. Germination and radicle growths were recorded after 72 hours at 25°C.

b. Effect of litter

One-gram litter was uniformly spread in petridishes following bed bioassays of Hussain *et al.* (1987, 1989 & 1994). The seeds of above mentioned test species were sown. They were incubated at 25°C for 72 hours

c. Root exudates bioassay

Mature plants of *A. viridis* were rooted out, washed with water and planted in a sterilized glass vials containing Hoagland solution. Glass vials were plugged with cotton and wrapped with brown papers. After 1 week incubation at 25°C, *Amaranthus* plants were removed and solutions from three vials were mixed. These *Amaranthus* – affected solutions were used as growth medium for the same test species as before.

a. Simulated rain leachates

Simulated rain leachates from *Amaranthus* were obtained by using 20 gm crushed *Amaranthus* shoots following methods of Hussain and Abidi (1991). These leachates were tested for their phytotoxicity against the same test species as described by Hussain and Abidi (1991).

b. Effect on cell development

Seeds of *Zea mays*, *Setaria italica* and *Pennisetum americanum* were grown in the extracts of as before. Root tips of the seedlings were treated with concentrated chloralhydrate for 12 hrs. The size and number of cells from 3rd to 5th cortical layer were determined following Hussain *et al.* (1984 & 1994)

Statistical Analysis

The data was statistically analyzed using *z*-test for seed germination and factorial analysis for other tests and significant differences were accepted at $p = 0.05$.

RESULTS AND DISCUSSION

1. Germination studies

The seeds either did not germinate or had less than 10% germination after 5 and 10 days at all the tested temperature. However, after 15 days the germination was 48% at 25, 40% at 30°C and 24% at 35°C. Many workers (Hussain *et al.*, 1980 & 1993; Hussain and Nasrin, 1985; Hussain and Ilahi, 1988; Durrani *et al.*, 1996) reported 25 - 30°C to be optimum for the germination of many plants. Hussain *et al.* (1980) observed that *Datura* seeds germinated better at 15 - 20°C. *Peganum harmala* seeds germinated better at 25 - 30°C (Hussain and Nasrin, 1985). The present findings agree with them. Mechanical scarification enhanced the germination to 55 – 60 % at 25°C and 30°C after 15 days. *Amaranthus* seeds have hard seeds coats, which probably impede germination. Hussain *et al.* (1980) stated that removal of testa promoted the germination of *Datura* seeds.

Table 1. Effect of sowing depth on the germination of *Amaranthus viridis*.
(Each value is a mean of 10 replicates, each with 10 seeds)

Observation Days	Soil Depth (cm)					
	4		8		12	
	Control	Treated	Control	Treated	Control	Treated
5	10	14	6	11	3	7
10	24	29	18	18	10	10
15	52	56	31	34	14*	21*

* Significantly diffused at p = 0.05 of Z test when compared with 4 cm depth

Soil depths to which seeds are sown affect the germination. It was seen that treated seeds had slightly better germination than non-treated seeds at the same sowing depth. Both in treated and non-treated seeds germination got delayed and decreased with increased sowing depth (Table 1). Similar findings have been reported for other species (Hussain and Ilahi, 1988, Hussain *et al.*, 1993). Our findings agree with them. Deep sowing prevents emergence of seedlings and reduces germination. Soaking the seeds in water at room temperature or in boiling water did not improve the germination. Similarly, application of various nutrient solutions, sodium hydrochloride, acetone H_2SO_4 alone or in combination with mechanical scarification failed to augment germination (Table 2) in this case. However, other workers (Hussain and Ilahi, 1988 & 1995; Hussain and Nasrin 1985) invariably reported that such treatments promoted the germination in many seeds. Our findings agree with those of Hussain & Ilahi (1988 & 1995) and Hussain & Nasrin (1985) who observed that water soaking was ineffective for the promotion of germination.

2. Effect of various levels of water

Soil moisture regulates germination as seeds failed to germinate under drought (1/4 FC) conditions. Whereas only up to 20% germination was achieved at field capacity and ½ capacity. Similarly, water - logged condition (1 ½ FC and 2 X FC) also reduced the germination to less than 8%. Seeds must imbibe ample water for softening seed coat and testa to be able to germinate. Water logged conditions prevented germination due to oxygen deficiency and poor gaseous exchange. Both water - stress and water - logged conditions were unfavourable for *Amaranthus* seeds.

3. Field study on growth

i Effects of various levels of water

The number of leaves and branches decreased with increased water stress whereas height of plant increased at ½ FC but declined drastically at severe stress (1/4 FC). However, there was no effect on the length of roots in water stressed condition (Table 2). The fresh and dry weight of leaves and shoots declined under water stressed condition, while that of roots generally increased (Table 2) The moisture contents of leaves, shoots and roots dwindled in stressed conditions compared to control. Likewise chlorophyll *a* and *b* and total chlorophyll also decreased severely in water - stressed plants. The poor over all stunted growth might be due to impaired internal water balance and poor chlorophyll contents. Many other studies (Mubarak *et al.*, 1982) have shown that water stress restricts the growth performance of plants.

Water - logged soils are inhospitable for mesophytes. Although, the number of leaves and branches decreased in water - logged conditions (Table 2), but the height of shoots, length of floral axis and roots and their fresh weights generally increased. This might be due to more absorption of water, which resulted in succulence of plants. However, the dry weight declined severely in water - logged (2 X FC) (Table 2) soils. The total chlorophyll contents increased in waterlogged conditions. This might be a response for physiological adjustment. The plants had more chlorophyll to compensate for the impaired functions of roots in waterlogged conditions. It was interesting to see that under water - stressed conditions chlorophyll contents declined where as in waterlogged conditions they increased.

ii. Effect of different light intensities

The number of leaves and height of plants increased in diffused and partial light than in the full light. The reverse was true for number of branches (Table 3). The length of roots and inflorescence showed insignificant variation in 3 light conditions. The fresh weight of leaves and shoots was reduced under full light (Table 3). Dry weight of leaves remained unaffected, but those of shoots and roots enhanced in full sunlight (Table 3). Moisture contents of leaves, shoots and roots declined in full light than in shady conditions. The total chlorophyll *a* and chlorophyll *b* increased in shade grown plants, while the reverse was true for chlorophyll *a* (Table 3). Light is an important factor governing the growth and distribution of plants as it controls the upper and lower limits of distribution. Within a microhabitat light intensity affects the growth of plants as observed in the present study.

Plants were generally taller and weaker in shady environment and had lower dry weight. Plants grown under full light had more dry weight and less moisture to make them more sturdy. The total chlorophyll *a* and chlorophyll *b* contents were high in shade grown plants. This is a physiological adjustment as shade plants generally have high chlorophyll *a* and chlorophyll *b* to compensate for the low light intensities.

Table 2. Effect of various levels of water on the growth performance of *Amaranthus viridis*

(Each value is a mean of 5 replicates, each with 4 plants)

Plant parts	FC	½ FC	¼ F	1 ½ FC	2 FC
	a.	No of leaves/branches			
Leaves	28a	18b	15b	17b	15b
Branches	7a	4b	4b	5b	4b
	b.	Height/length (mm)			
Shoot	102a	199a	146b	152b	247c
Root	125a	120a	121a	134a	130a
Infl	70a	95b	76a	35c	82b
	c.	Fresh weight (mg)			
Leaves	1.0a	0.9a	0.6a	1.1a	1.4a
Shoot	58a	42b	30c	90d	100d
Root	17a	19a	19a	20a	60b
	d.	Dry weight (mg)			
Leaves	0.2a	0.3a	0.3a	0.2a	0.2a
Shoot	23a	20a	16b	24a	15b
Roots	5a	6a	9b	5a	4a
	e.	Moisture Contents			
Leaves	400a	200b	100c	450b	600d
Shoot	154a	111b	85c	165a	186d
Roots	273a	197b	109c	300a	456d
	f.	Chlorophyll mg/l			
Total chl.	17.9a	14.9b	5.5c	25.2d	19.1a
Chl – *a*	12.7a	6.3b	2.2c	10.4a	13.7a
Chl – *b*	25.97a	8.7b	9.2a	14.8c	27.8a

Figure within a row followed by the same letter (s) are not significantly different from each other at p = 0.05.

Table 3. Effect of different light conditions on the growth and performance of *A. viridis*
(Each value is a mean of 5 replicates, each with 4 plants)

Plant parts	Shade	Partial light	Full light
	a.	No of leaves/branches	
Leaves	37a	27b	14c
Branches	10a	5b	14c
	b.	Height/length (mm)	
Shoot	255a	230a	188b
Root	125a	107b	121a
Infl.	80a	57b	91c
	c.	Fresh weight (mg)	
Leaves	1.9a	1b	1.2b
Shoot	80a	70b	60c
Root	20a	18a	22a
	d	Dry weight (mg)	
Leaves	0.5a	0.4a	0.5a
Shoot	29.6a	34.3b	30b
Roots	4.2a	4.5a	10b
	e.	Moisture Contents	
Leaves	280a	150b	140b
Shoot	170a	78b	66c
Roots	376a	308b	120c
	f.	Chlorophyll mg/l	
Total chl.	44a	40.7b	33.5a
Chl – *a*	13.5a	12.7a	12.1c
Chl – *b*	30.1a	28.3a	21.4b

Note: Figures within a row followed by the same lette: (s) are not significantly different from each other at p = 0.05

4. Allelopathic potential

Cold and hot water extracts from fresh shoots suppressed the germination of *Setaria* and wheat. While cold and hot water extracts from dried shoots retarded germinating of all the 3 crops except maize (Table 4). Many weeds allelopathically suppressed the germination of test crops. Similarly the germination of test plants was reduced by aqueous extracts from *Euphorbia*, *Avena*, *Cynodon* and *Phalaris* fresh and dried parts (Hussain *et al.*, 1980 & 1993; Hussain and Nasrin, 1985; Hussain and Ilahi, 1988; Durrani *et al.*, 1996) and our findings agree with them. The germination inhibition is a species specific process as reported by other workers (Rice, 1974; Hussain *et al.*, 1980 & 1993; Hussain and Nasrin, 1985; Hussain and Ilahi, 1988; Durrani *et al.*, 1996).

The radicle growth of all test species except that of *Setaria* and maize in cold water extract from fresh parts was demonstratably inhibited (Table 4) in all the treatments especially in hot water extracts and our findings are supported by Inam *et al.* (1989) and Hussain *et al.* (1989) who reported inhibitory effects of other weeds. Hot water extracts of *Phalaris* and *Cannabis* retarded the growth and germination of test species and our findings agree with them.

Plant litter releases phytotoxic substances before their complete decay. This was true in the present case as seeds grown directly over moist litter beds exhibited reduced germination with the exception of pearl millet. Similarly, the seedling growth of all test species was retarded by litter (Table 3). Litter from many other plants (Hussain *et al.*, 1987; Hussain and Abidi, 1991) had been shown to be allelopathic and our findings are in line with them. Germination of test species did not reduce in simulated rain leachates.

However, radicle growth of all tested species was suppressed (Table 4). The toxicity of simulated and natural rain leachates has been well demonstrated by many workers (Hussain and Abidi, 1991). The present findings also suggest the toxic effects of simulated rain leachates from *Amaranthus*.

Many plants exudates toxic substances from roots into the soil (Rice, 1984). Roots of *Amaranthus* released toxins into the growth medium that retarded the germination of the test species and radicle growth of *Setaria* and *Brassica* (Table 4). Dirvi and Hussain (1979) observed that *Dichanthium* release toxic root exudates. Similarly *Setaria* (Hussain *et al.*, 1984) also known for toxic root exudates, and our results agree with them. The retarded radicle growth might possibly be due to either loss of cell division or retarded development or both as reported by Hussain *et al* (1984 & 1994). The extract suppressed the development of meristematic cells as treated cells had small size than the control (Table 5). There were more cells/unit areas of the treated root tip than in the control, which had large size cells. Hussain *et al.* (1984 & 1994) also reported the suppression of cell development by *Eragrostis* and *Imperata*. The present also agree with them.

The present study concludes that *A. viridis* seed might germinate better within a range of 25 – [illegible] in superficial layers. It is intolerant to salinity, water stress and water logging. It preferred certain light conditions. The plant has allelopathic potential, which operates through release of phytotoxins during water soaking, root exudates and by rains.

Table 4. Effect of cold and hot water extracts and litter on the germination, radicle growth of test species, each value, expressed as % of control, is a mean of 5 replicates, each with 10 seeds.

Test Species	Fresh parts		Dry parts		Litter bed
	Cold extract	Hot extract	Cold extract	Hot extract	
a. Germination					
Pennisetum americanum	100	90.8	80.8	[illegible]	[illegible]
Setaria italica	35.4**	12.5**	43.3**	[illegible]	[illegible]
Zea mays	[illegible]	30.6*	97.6	[illegible]	[illegible]
Triticum vulgare	80.43	74.3*	80.4	[illegible]	[illegible]
b. Radicle growth					
Pennisetum americanum	62.2*	50.5**	4.5**	[illegible]	[illegible]
Setaria italica	125	12.5**	38.**	[illegible]	[illegible]
Zea mays	114	79*	124	37.0**	[illegible]
Triticum vulgare	58.2**	23.8**	13.5*	12.2*	[illegible]

* & ** significantly different from control at p = 0.05 and 0.01, respectively

Table 5. Effect of *A. viridis* extract on the development of root tip, cell of test species (Each value is a mean of 10 replicates).

Test species	Cell size			Cell number		
	Control	Test	% of control	Control	Test	% of control
Pennisetum americanum	1.6	1	62.5**	11.5	12.1	105.2
Setaria italica	1.4	1	71.4**	9.5	10.5	110.5
Zea mays	1.9	1.1	57.9**	12.5	14	112

** significantly different from control at p = 0.01

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