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AGE ENVIRONMENTAL FACTORS, GERMINATION AND SEEDLING EMERGENCE OF *OCIMUM BASILICUM* L

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ABSTRACT

Fresh and exhumed seeds of *Ocimum basilicum* L. gave 100 % germination at 13/11-h alternating temperature of 20/10, 32/18, 38/24 and 40/32 °C but did not germinate at constant temperatures. Germination was prevented in the dark but occurred with a 13-h photoperiod. With increasing age, seed dry weight was reduced progressively as with speed and extent of germination; seeds 5 or more years old were not viable. There was 90% emergence from seeds sown on the soil surface but only 33% produced seedlings when buried 2 mm deep and emergence was 20% or less from those sown at depths of 5 mm or more. Mucilage by the seeds apparently helped to prevent desiccation. The ecophysiological significance of the result is discussed in relation to weed control in the Sudan Gezira.

INTRODUCTION

Ocimum basilicum L. (Labiatae), is a common annual weed in the clay soils of central Sudan. It displays no seasonal periodicity in germination or emergence and germinates whenever soil moisture is adequate (Babiker, 1979; Babiker & Ahmed, 1986). Because of this and with a relatively high tolerance to herbicides, *O. basilicum* is a problematic weed in all crops grown in the area including cotton, groundnuts, sugar-cane and vegetables (Hamdoun & Babiker, 1978; Ishag, 1979; Babiker, 1979; Babiker & Ahmed, 1986).

Little is known of the germination behaviour of *O. basilicum* and a study was made of several relevant factors to provide a basis for more efficient control.

MATERIALS AND METHODS

General

Growth-cabinet, glasshouse and field experiments were undertaken to study the effects of temperature, light, seed age and burial depth on *O. basilicum* seed germination and establishment. Seeds were collected from the Gezira Research Farm, Wad Medani, Sudan, in 8 different years (January 1970 - January 1988) and seed lots of 1970, 1980, 1982 and 1983 were stored in paper bags in the dark at temperatures ranging from 23-32 °C. *O. basilicum* seeds, which were harvested in 1984, 1986 and 1988 were placed in fine mesh cloth bags (100 seeds/bag) and the bags were buried to a depth of 3-5 cm in Gezira soil in pots (30 cm i.d). The pots were left under natural conditions of the Gezira.

Growth-cabinet experiments were conducted at the University of Qatar in a Trembath TS9 Environmental Controller (Surrey CR9 4BT, England) with light intensity at seed level of 34.0 μ mol $m^{-2}s^{-1}$ corresponding to 3% of full sunlight (1230 μ mol $m^{-2}s^{-1}$) and a 13-h photoperiod at alternating temperature regime of 32 °C during day and 18 °C during night. The relative humidity in the chamber was 40-60 %. These conditions approximated to those prevailing in the fields of the Gezira during July-August when most crops were being sown. All germination tests were carried out in 9-cm Petri-dishes each lined with 2 Whatman No. 1 filter paper to which 5-6 cm^3 of distilled water was added. There were 4 replicate dishes each of 50 seeds arranged in randomized blocks. The tests lasted for 7 days and radicle protrusion through the seed coat was the criterion for germination.

Experimental details

Freshly-collected seeds of *O. basilicum* of 1984, 1986 and 1988 respectively were tested immediately after harvest for germination. The Petri-dishes were arranged randomly in blocks, the experimental design being 3 populations x 1 treatment x 4 replicates.

The water content of seeds was determined using 4 replicates (25 seed each) from each of the 8 different-aged populations. The seeds were weighed before and after attaining constant weight at 100 °C for 6h. A linear regression of fresh versus dry seed weights was calculated.

Seeds of different ages (Table 1) were exposed to alternating light and dark or to complete darkness. Dishes for the dark treatment were wrapped individually in black plastic sheets and germination assessed only on the last (7th) day. The experimental design was an 8 x 2 factorial with 4 replications.

Seeds of 1984, 1986 and 1988 respectively were tested for their speed of germination. Counts were made daily for 7 days to determine the time to attain 50 % germination.

For the remainder of experiments described below, the 1988 seeds population was used. Germination tests were conducted at 4 day/night temperature regimes (20/10, 32/18, 38/28 and 40/32 °C) selected to simulate thermal conditions which may prevail in the Gezira during summer, autumn and winter. Tests were also carried out at 4 constant temperatures (20, 30, 40 and 50 °C).

O. basilicum seeds were planted at 0.0, 0.2, 0.5, 1.0, 2.0, 3.0 and 4.0 cm depth in sterile Gezira soil in 6 x 10 cm black plastic pots in naturally lit greenhouse in the Gezira Experimental Station during August 1988 (day/night temperature 34/26 °C). There were 4 replicates of each treatment arranged in a randomized block design and 100 seeds per pot. Sub-irrigation was adopted so that the soil surface would not be altered. Emergence was recorded daily for 7 days after which emerged seedlings were pulled out. The soil in each pot was then washed through a fine mesh sieve and *O. basilicum* seeds were recovered and examined for radicle protrusion.

Two experiments were conducted to study the influence of storing the seeds in the field on germination and seedling emergence. In the first experiment, and at 3 month intervals, some of the seeds stored in the field were returned to the laboratory, removed from their bags and tested for their germinability under dark/light or dark conditions (see Methods).

In August 1989 the second experiment was set up under natural conditions (12-h photoperiod with a maximum midday photosynthetically active photon flux density of 1300-1400 μ mol $m^{-2}s^{-1}$ and a day/night temperature range of 38/27 °C). Generally 60 seeds of *O. basilicum* were sown in small plastic cups (6.7 cm i.d) with 100 g of Gezira soil and subirrigated. Unless stated otherwise emergence of seedlings was recorded daily for 7 days. The following treatments were imposed: (a) seeds were sown at depth of 4 cm, (b) seeds were sown on the surface, (c) seeds were sown on the surface and the cups were covered with black plastic sheets (dark treatment) for 4 days, the number of emerged seedlings were counted following the removal of the black cover and (d) ungerminated seeds from treatment (c) were allowed to germinate under natural conditions (black cover preventing light was removed) for 3 days. In both experiments the treatments were arranged in a randomized complete block design with 4 replicates.

Seeds (200) were placed in 4 Petri-dishes and water was added as already described. After 2-3h seeds were removed and put on dry filter papers in dry Petri-dishes, i.e. the seeds were left with their mucilage intact. Two days later water was added and the progress of germination determined.

The effect of different factors affecting germination and seedling establishment were compared by analysis of variance.

RESULTS

Seed dormancy

Freshly harvested seeds of 1984, 1986 and 1988, respectively, were non-dormant and final percentage germination was invariably 100 %.

Effect of age on seed weight

The weight of a single seed of *O. basilicum* consistently declined with age from 1.11 mg (1988 population) to 0.81 mg (1970 population), a loss in weight of 27 %. For all seed populations the fresh weight differed significantly ($P<0.5$) from the dry weight. It can be seen that the loss in weight (estimate of the moisture content of seeds) was between 2.5 % for 1970 population to 6.1 % for 1988 population respectively (Table 1). For the entire range of seed age, fresh seed weights (x) were correlated with dry seed weights (y); ($Y = 2.1063 + 0.8533x$; $r^2 = 0.914$). The difference in seed weight was therefore not due to water content of seeds.

Effect of age on germination in light and dark

The responses were dissimilar for the 2 experiments (Table 1). It can be seen that light was essential for the germination of *O. basilicum* seed. Seeds 5 or more years old were non-viable and this was confirmed by fusicoccin and tetrazolium tests.

Table 1

Effect of seed age on fresh weight and percent germination (tests were run during 1988, ± one SE)

Year of collection	Fresh weight of 100 seeds mg	% Loss	Dry weight of 100 seeds mg	% Loss	% Germination Dark	% Germination Light/Dark
1988	111.0 ± 3.1	--	104.3 ± 2.0	6.1	0.0	100.0 ± 0.0
1986	94.0 ± 2.8	15.0	90.1 ± 1.0	4.1	0.0	90.0 ± 3.0
1984	91.0 ± 0.0	18.0	86.9 ± 1.7	4.5	0.0	76.0 ± 4.0
1983	91.0 ± 0.9	18.0	88.0 ± 1.6	4.2	0.0	0.0
1982	87.0 ± 0.8	21.7	83.7 ± 2.0	3.8	0.0	0.0
1980	85.0 ± 0.5	23.4	82.3 ± 1.8	3.2	0.0	0.0
1972	86.0 ± 1.2	22.5	82.9 ± 1.1	3.6	0.0	0.0
1970	81.0 ± 0.9	27.0	79.0 ± 0.0	2.5	0.0	0.0

Effect of age of seed on the speed of germination

Fig. I shows the periods of incubation required by the seeds to attain 50% germination. Germination occurred for a longer period of time for seeds collected in 1984 and 1986 relative to those from 1988. Seeds which were 2-4 years old had slower germination rates and finished with average total germination under 85%.

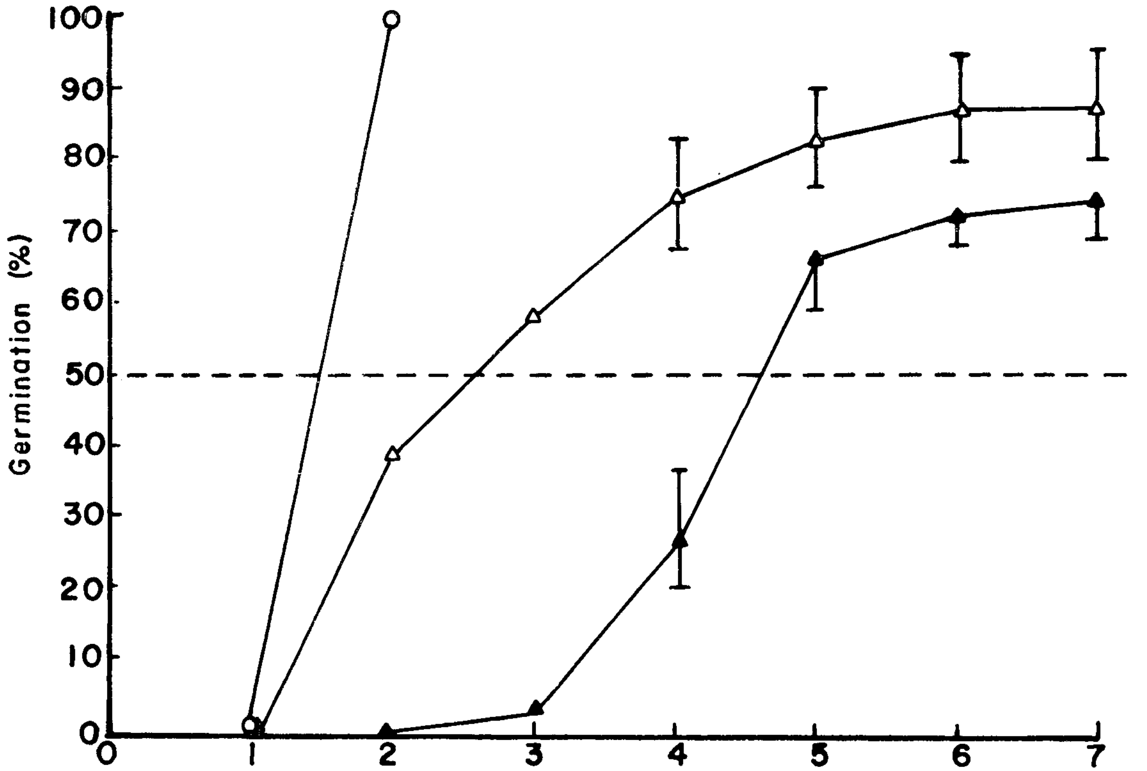

Fig. 1: Effect of age on the speed of germination ○———○ 1988 seeds △———△ 1986 seeds ▲———▲ 1984 seeds. Vertical bars indicate ± one SE.

Effect of alternating and constant temperatures

Though the seeds of *O. basilicum* germinated to 100% over the whole alternating temperature regimes tested, constant temperature prevented germination (Fig. 2).

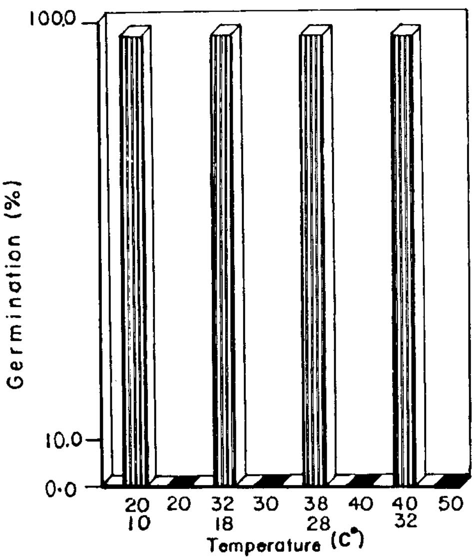

Fig. 2: The effect of alternating ▥ and constant ■ temperature on germination of *O. basilicum* seeds.

Emergence of seedlings

Successful emergence of seedlings occurred at 0.0-0.2 cm and an increase in depth of sowing drastically ($P<0.001$) reduced emerging seedlings from other depths. At the end of the experiment 2-4 % of the sown seeds (unemerged as seedlings) showed radicle protrusion (Table 2).

Table 2

The effect of depth of sowing on the emergence of seedling and total germination of *O. basilicum* (emerged and unemerged seedlings) (± one SE)

	Depth of sowing (cm)						
	0.0	0.2	0.5	1	2	3	4
Emergence (%)	90.0±9	33.0±10	18.0±8	20.0 ± 6	18.0 ± 5	10.0 ± 8	0.0
Total (%) germination (emerged and unemerged seedlings)	90.0±9	37.0±10	20.0±8	22.0 ± 5	22.0 ± 6	13.0 ± 6	0.0
Unemerged* seedlings (%)	0.0	4.0	2.0	2.0	4.0	3.0	0.0

⋆ Unemerged seedlings = Total germination minus emerged seedlings

Phenological studies of buried seeds

The 1988 seeds stored in the field showed 95-100 % germination under dark/light treatment and germination was prevented under dark conditions during the period March 1988 - June 1989 (Fig. 3).

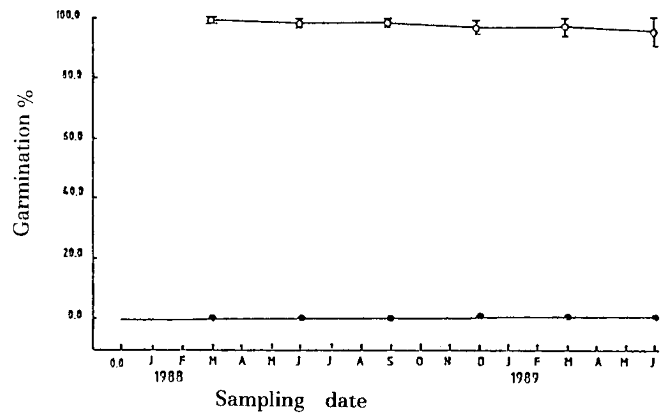

Fig. 3: Germination at alternating temperature 32/18°C in a light/dark (o) or complete darkness (●) of *Ocimum basilicum* seeds when removed from the field at different times after burial in January 1988 (± one SE).

In the second experiment, seedling emergence was affected by depth of burial in the soil and by absence of light (Table 3).

Table 3

The percent emergence of seedlings of *O. basilicum* sown at diffirent depth and subjected to light/dark or complete darkness (mean of 4 replicates ± one SE).

Treatment	Emergence %
a - seeds were sown at depth of 4 cm	0.0
b - seeds were sown on the surface (0 cm)	100.0 ± 0.0
c - seeds were sown on the surface and cups were covered to prevent light for 4 days	10.0 ± 0.8
d - black cover preventing light in treatment (c) was removed and the experiment was allowed to continue for a period of 3 days	67.0. ± 1.25

The effect of mucilage on germination

A common feature among all seed lots was that 5 minutes following imbibition all seeds were surrounded by halos of drying mucilage. Two days following the imposition of dry conditions, 10-22 % of *O. basilicum* germinated; after further addition of water germination was completed to 100 %.

DISCUSSION

The influence of factors such as seed age was found (Roberts, 1972; Powell & Mathews, 1977) seriously to affect and alter the permeability characteristics of cell membranes in seeds. The results show that the germination capacity of different-aged populations was significantly depressed with an increase in age and that seeds more than 5-6 years are unviable, and the differences in seed weight reflect differences in the amount of reserve substances presumably lost due to respiration and not to seed water content (Wulff, 1986). Brown & Mayer (1986) pointed out, that for any species, if germination is delayed or proceeded slowly it indicated reduced vigour. Like *O. americanum* L. (Varshney, 1986) *O. basilicum* does require light for its germination.

The effects of alternating temperatures as opposed to constant ones are: some seeds remain dormant when held at constant temperatures and, secondly, exposure to alternating temperature regimes increases the maximum fraction that germinates in a population (Garcia-Huidobro, Monteith & Squire, 1982b). The results of this study show that the seeds of *O. basilicum* germinate only under alternating temperatures. Roberts & Boddrell (1985) experimenting with the seeds of the annual weed *Aethusa cynopium* L. found that at constant temperatures none of the intact seeds germinated, whereas at alternating temperatures there was some germination of the seeds. Froud-Williams, Hilton & Dixon (1986) found that all the seeds of *Poa trivialis* germinated when incubated in light at alternating temperatures, while germination failed to occur at constant temperatures in the absence of light.

For an annual like *O. basilicum,* seedling establishment is of vital importance for population maintenance. The results illustrate that *O. basilicum* seedlings only emerge when the seeds are sown on the surface of the soil, and that almost no seeds showed radicle protrusion as sown in deeper depths. Field observations, during August 1988 in a cotton field in the Gezira, on 20 seedlings of *O. basilicum,* showed that none of them emerged from below 2 mm. The emergence from shallower depths may be explained by the observation that, as the seeds are sown on soil surface they are exposed to direct sun (light requirement) and dry air, i.e. the seeds are exposed and adapted to a wide range of environmental (thermal) conditions, and therefore they germinate (Ismail, 1985). However, when the seeds were buried they were deprived of light, ad thereby they do not germinate. This finding concurs with Thomson (1974) and Thompson, Grime and Mason (1977) who indicated that the enhancement of germination, by alternating temperatures, can be interpreted as inhibiting germination of buried seeds and promoting germination of seeds at or near the soil surface.

Thompson and Grime (1979) described the characteristics of seeds which form persistent seed banks as having very small seeds (weight/seed<2 mg). The $\log_{10}$ seed weight class of 1984, 1986 and 1988 respectively lies within the range of species whose seeds persist in cultivated soil for at least five years (Thompson, 1987). Storage of seeds in the laboratory bear little resemblance to conditions experienced by seeds in their natural habitats. The phenological studies (Baskin & Baskin, 1981) substantiated the laboratory results indicating that *O. basilicum* seeds did not change in their germination responses during their storage in the field - at least for 1-2 years after the seeds were shed - and provided a firmer basis that light was a basic requirement for germination and seedling emergence. Karssen (1981) pointed out that burial of weed seed in soil may lead to alleviation or re-alleviation of dormancy.

Mucilaginous species prevail in the families Cruciferae, Euphorbiaceae, Labiatae, Leguminosae, Onograceae, and Plantaginaceae (Young & Evans, 1973). For *O. basilicum* seeds on the soil surface, the mucilage (a) prevents seed desiccation by discouraging water loss through evaporation, (b) establishes firm contact between the soil particles and the seed, and (c) acts as a wick, absorbing moisture from the surroundings. Harper and Benton (1965), and Young and Evans (1973) have shown how seed mucilage might provide a shield against moisture vapour loss to the atmosphere.

From this study, viable *O. basilicum* seeds were found to germinate and establish within 7 days. Under tropical conditions, individuals taking longer than 7 days to germinate would have little chance of survival in a crop (Garcia-Huidobro, Monteith & Squire, 1982a; Ismail, 1985). With frequent soil disturbance (see Solbrig et al., 1988 for definition of disturbance) associated with arable cultural practices, a natural *O. basilicum* infestation will result from seeds of a range of ages between 1-5 years. Seeds of *O. basilicum* having been released from enforced dormany, i.e. are put on surface of soil, and as favourable germination conditions prevail (light and alternating temperatures), they can establish successful seedlings. If these seedlings are not killed by cultivation or herbicides and are allowed to become adult plants and shed seeds, this will result in the replenishing of the seed bank for up to a 5-year period.

The modes of coping with any weed problem can be grouped into three categories: (a) acceptance of yield loss, (b) assessing quantitatively how much is the loss, and (c) development of weed control programmes designed to eradicate or to minimize future invasion of this weed. Upshot of this research provides insight into the germination ecology of *Ocimum basilicum*. Methods of stimulating germination before crop sowing (e.g. prewatering or heavy rainfall) which result in rapid depletion of the seed reservoir, may have practical applications for crop yield increases in areas dominated by *Ocimum*. Furthermore, in the light of the relatively large number of annual weeds invading crop plants in the Gezira, it would appear prudent to investigate whether the relationship between germination behaviour and seed age observed in *O. basilicum* also exists in other weedy species.

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تأثير عمر البذور وعوامل البيئة على انبات وخروج بادرات نبات الريحان

أحمد محمد علي اسماعيل و فتحي محمد خليفة
و عبد الجبار الطيب بابكر

تمت دراسة تأثير أعمار مختلفة لبذور الريحان - نبات منافس وضار بالمحاصيل - على الانبات تحت ظروف بيئية مختلفة . أجريت التجارب على البذور التي قطفت من النبات الأم - على مدى ثلاث إلى أربع سنوات - في مقابل التي وضعت في أكياس وخزنت في الأرض لكي تتلاءم مع الظروف داخل بيئة التربة ومتابعة هذه البذور بنبشها في شهور مختلفة . كما درس الباحثون أثر الضوء ودرجة الحرارة الثابتة والمتناوبة وعمق مكان البذور على انباتها .

أكدت الدراسة أن بذور الريحان تتنبت فقط على سطح التربة وفي وجود الضوء وعلى مدى واسع من درجات الحرارة المتناوبة (١٠ ـ ٢٠ ، ١٨ ـ ٣٢ ، ٢٤ ـ ٣٨ ، ٣٢ ـ ٤٠ °م) . كما أوضحت الدراسة أن البذور تموت اذا خزنت في بنك البذور الأرضي لأكثر من أربعة أعوام حيث أن البذور تفقد قدراً كبيراً من حيويتها مع زيادة عمرها . كما أوضحت الدراسة أن المادة الهلامية التي تحيط بالبذور عندما تستنقع تزيد من قوة امساكها للرطوبة وتساعدها على الانبات .

يستخلص الباحثون أن مكافحة نبات الريحان كنبات منافس للمحاصيل الزراعية بمشروع الجزيرة يمكن أن يتم حينما يستحث ويستنشط استنبات بذور الريحان بواسطة الري الصناعي أو الأمطار - قبل زراعة المحصول - وذلك لابادة أكبر نسبة من بادرات الريحان قبل أن تدخل في طور الازهار .