DOI: https://doi.org/10.36811/ijpsh.2021.110032 **IJPSH: November-2021: Page No: 58-67**

International Journal of Plant Science and Horticulture

Research Article **Open Access**

Biological activity of *Arum Cyreniacum* (Araceae) and potential use as Bioherbicide

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Received Date: *Oct 08, 2021 /* ***Accepted Date:*** *Nov 22, 2021 /* ***Published Date:*** *Nov 24, 2021*

Abstract

The present investigation aims was carried out to study the biological activity of aqueous extract and shoot crude powder of *Arum cyreniacum* (ACSAE and ACSCP) on some germination and growth parameters (germination bioassay experiment) besides major physiological, and biochemical processes (pot experiment) In *Hordeum vulgare* (crop species) and Phalaris minor (weed species) of different concentrations of *A. cyreniacum* on germination percentage (GP), coleoptile (CL) and radicle (RL) lengths, seedling shoot and root length seedling fresh and dry weight, some nutrients (N, K, Na, Cu, Fe, and Ni), and photosynthetic pigments .Generally, the effect of the all concentrations levels of the extract on (GP), (CL) and (RL) on *H. vulgare* seeds was ineffectively decreased with increasing the concentrations of *A. cyreniacum* while the percentage was significantly decreased with increasing the concentrations of the with *P. minor.* All concentrations levels of the extracts reduced coleoptile (CL) and radicle (RL) lengths of *H. vulgare*. Likewise, the reduction in the two parameters was documented in *P. minor*. Were more affected in P. minor compared to *H. vulgare*. There was a significant within the concentration of micronutrients as well as the entire photosynthetic color substance of P. minor seedlings grown in ACSCP. Treatment with ACSCP had a more negative effect on total nitrogen in *P. minor* than on *H. vulgare.*

Keywords: R Hordeum Vulgare; Growth Parameters; Nutrient Content; Photosynthetic Pigments

Cite this article as: Ahlam K Alaila, Sami M Salih, Ahmed A Abdulrraziq. 2021. Biological activity of *Arum Cyreniacum* (Araceae) and potential use as Bioherbicide. Int J Plant Sci Hor. 3: 58-67.

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Introduction

Allelopathy is a physiological process with ecological implications [1]. A living being produces one or more biochemical (allelochemicals) that impact the development, survival and generation of other living beings. It also involves chemical interactions at all levels of complexity, from microorganisms to higher plants [2]. When plants are exposed to allelochemicals, their growth and development are affected [3]. Weeds are known to cause enormous losses for a large number of field crop species due to their interference in agroecosystems [4]. They have a significant economic impact on agricultural production [5] as evidenced by the efforts spent on their

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management. The reduction in crop yield may also be attributed to the allelopathic property exhibited by a number of weeds, especially, the aggressive ones. Such species of weeds, because of their growth habit, make agricultural operations more difficult. Furthermore, the crop contaminated with the weed seeds, is considered to be of poor quality such as seeds of Avena fatua and Phalaris minor in wheat and barley seeds [6].

The biological solution to minimize the perceived hazardous impacts from herbicides and insecticides in agriculture production lies in the field of allelopathy. The harmful impact of allelopathy can be exploited for pest and weed control [7,8]. Much inquire about has reported the potential of allelopathic plants to influence weed development. The address of what allelopathic plants ought to be chosen, how they are connected, and their benefits ought to be seen as an imperative some time recently presenting them to the agriculturists for field usage [9]. In this respect, utilize of crops having allelopathic properties can decrease the reliance on manufactured herbicides and increment edit yields [10]. Barly (Hordeum vulgare L Poaceae) is considered from the main cereal crops in libya. Management must be designed to find a long-term method of control of canary grass (Phalaris minor Retz. Poaceae). It is found predominantly in fields cultivated for barly. It is indigenous to the Mediterranean region and was introduced to Australia, Africa, Hawaii, India, and Pakistan and since then to many countries of the world [11]. Therefore, the purpose of the present study was to carry out an evaluation on the biological activity of Arum cyreniacum (Araceae) crude powder of their aerial shoot on some growth and physiological parameters of the most problematic weed in H. vulgare L. fields; *P. minor*. We hope that the study will provide information about the possibilities of using the donor specie as bioherbicides.

Materials and Methods

Plant materials and experimental design.

Shoots of the donor specie *Arum cyreniacum* (ACSCP) have been collected from algabal alakhdar (Libya) during the vegetative stage. The plant materials were dried in shade then ground in a Wiley Mill to coarse uniform texture and stored in glass jars until use. Seeds of the weed (*Phalaris minor*) and crop species (*Hordeum vulgare* cv. alhsad 176) were purchased from the Department of Plant Crops, College of Agriculture, and Omar Al-Mukhtar University, Libya. Stock aqueous extracts and subsequent dilutions were obtained by the following methods:

Dried powders of shoots of the target specie (75 g for each) were extracted with 1000 ml distilled water. The extract was conducted in dark for 24 h at 25ºC. The supernatant was taken and centrifuged at 3000rpm for 15 minutes; this would be full strength concentration (100%). The extracts were prepared no more than 48 h in advance and were kept in a refrigerator at 5ºC until used and the purified extract was adjusted to pH 6.8 with 1M HCl. Series of dilutions were prepared from the stock solutions (10, 20and 40% besides the control) for *Arum cyreniacum* (ACSAE) were tested for their effects on germination parameters, and seedling growth of *Phalaris minor* besides the crop species

Germination Bioassay

Petri-dish experiment was applied to investigate the biological activities of the target species aqueous extracts on germination percentage (GP), and coleoptile (CL) and radicle (RL) lengths of *Hordeum vulgare* (crop species) as well as the weed speceies (*Phalaris minor*). To achieve this experiment, ten seeds of each of the weed and crop species were arranged in 9-cm diameter Petri-dishes lined with two discs of Whatman No.1 filter paper under normal laboratory conditions with day

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temperature ranging from 19-22oC and night temperature from 12-14oC. 10 ml of the respective target species aqueous extracts (10, 20 and 40%) or distilled water as control were added daily to three replicates in a randomized complete block design for seven successive days. Before, the seeds were immersed in 2% CHLOREX for 2 minutes then rinsed four times with distilled water. Finally, the seeds were soaked in aerated distilled water for 24 hours.

Pot Experiment

The soil samples were finally sterilized at (90ºC for 48 h) to remove any microorganisms and weed seeds. Twenty seeds of each of the weed and crop species were sown in plastic pots (16 cm in diameter) in pure culture practices with about 1000 g of sandy loam soil thoroughly mixed (w/w) with 1,2 and 3 % of electrically crushed crude powder of the shoots of the target species. One treatment was run as control with zero percent of crude powder. Treatments were arranged in a completely randomized block design with tow replicates. The plants were watered every two days on the average with normal tap water. The amount of water corresponding to average soil–plant evapotranspiration calculated from weight loss over a 24 –hour interval. The experiment was performed under normal laboratory conditions (20±2°C temperature, 75±2% relative humidity, and 14/10 h light/dark photoperiod).

After 21 days, the homogenous seedling was carefully collected from each treatment, washed with tap water to remove the adhering soil particles, and then, by distilled water, gently blotted with filter paper. The seedlings were separated into shoots and roots for the determination of seedling fresh weight as well as seedling length. Other samples were dried at 65oC till constant weight to determine the seedling dry weight.

Photosynthetic pigments

The photosynthetic pigments (chlorophyll a, chlorophyll b and carotenoids) were determined spectrophotometrically according to [12].

Determination of minerals

Seedlings were carefully and thoroughly cleaned, blotted dry between absorbing paper and their dry weights were measured after oven drying at 70°C for 72h.Oven dry samples of seedlings were finely ground and assayed for-mineral ion content by the wet digestion method (Humphries, 1956). Minerals (N, K, Na, Cu, Fe, and Ni) were determined using an atomic absorption spectrophotometer (Perkin-Elmer, 2380) and expressed on the basis of dry weight.

Statistical Analysis

Statistical analysis was performed using a computer run program (Minitab software). By ANOVA followed by Turkey's test was performed to show the statistical significance among the means of the groups. Results were expressed as mean ± Standard Division (SD). P-value below 0.05 was considered to be statistically significant.

Results

Germination Bioassay parameters

The germination percentage (GP), (CL), and (RL) of *H. vulgare* seeds was ineffectively decreasing with increasing the concentrations of ACSAE in Table 1. The percentage decreased from 100% at the control to 60% at 40% concentration level after seven days from sowing. The length was visibly reduced from 48.7cm for control to 18.3 cm at 40% concentration after seven days from the beginning of the experiment.

The corresponding allelopathic effects on radicle length (RL) were recorded. Data

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demonstrated that the RL decreased significantly upon applying different concentrations of the extract. The length reduced from 25.3cm at control to 8.3cm at 40% concentration level. The regression line between (CL, RL) and the different concentrations of the applied extracts confirmed the negative relationship between the two variables. Growth parameters. On the other hand, the germination percentage (GP), (CL), and (RL) of *P. minor* seeds was effectively decreasing with increasing the concentrations of ACSAE (Table 1). The percentage decreased from 100% at the control to 40 % at 20% concentration level after seven days from sowing. On the other hand, no the germination at 40% concentration. The length was visibly reduced from 31.7cm for control to 11.8 cm at 20% concentration after seven days from the beginning of the experiment.

The corresponding allelopathic effects on radicle length (RL) were recorded. Data demonstrated that the RL decreased significantly upon applying different concentrations of the extract. The length reduced from 12.7cm at control to 2.8cm at 20% concentration level. The regression line between (CL, RL) and the different concentrations of the applied extracts confirmed the negative relationship between the two variables. Growth parameters.

Table 1: Allelopathic effect of different concentrations of *Arum cyreniacum* shoots aqueous extract (ACSAE) on germination percentage (GP), (CL) and (RL)of *Hordeum vulgare* and *Phalaris minor* seeds experiments seven days after sowing. Data are means of three replicates.

Concentration (%)	**germination percentage (%)**	**Seedling's length (cm)**	**Coleoptile length (cm)**	**Radical length (cm)**
	Hordeum vulgare			
0	100	48.666±2.89^{a}	23.333± 1.528^{a}	25.333± 1.528 ^{a}
10	100	31.667±3.51^{b}	16.667± 2.52^{b}	15± 2.65^{b}
20	100	25.333±0.577^{c}	14±1.00^{bc}	11.333±1.155^{bc}
40	60	18.333±1.528^{d}	10±1.00^{c}	8.333± 0.577^{c}
	Phalaris minor			
0	100	31.666±3.79^{a}	19±2.65^{a}	12.666±1.528^{a}
10	80	16.833±1.607^{b}	10±1.000^{b}	6.833±0.764^{b}
20	40	11.833±0.764^{bc}	9±0.500^{b}	2.833±0.289^{c}
40	0	0	0	0

Data are expressed as mean ± SD of 3 replicate. Within each row, means with different superscript (a, b, c or d) were significantly different at $p<0.05$. Where means superscripts with the same letters mean that there is nonsignificant difference ($P>0.05$).

Growth parameters

The allelopathic effects of *Arum cyreniacum* (ACSCP) shoot crude powder of both on shoot and root lengths, fresh and dry weight of *Hordeum vulgare* and *Phalaris minor* seedlings are illustrated in Table 2. Increasing the concentration of ACSCP in the soil caused a gradual decrease in a total seedling length as well as shoot and root length of both treated plants and the reduction was much dramatic in *P. minor* (Table 2) was decrease of 80% in shoot length was exhibited at 10% concentration and reached 60% at 20% concentration.

At concentration 20%, a fresh weight of about 0.409g was achieved in *P. minor* seedlings. This accounts for a decrease of about 97% in the fresh weight compared to the corresponding control.

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Similarly, at concentration 20%, a dry weight of about 0.254g was achieved in *P. minor* seedlings. This accounts for a decrease of about 91% in the dry weight compared to the corresponding control while a fresh weight of about 0.114 g was achieved in *H. vulgare* seedlings. This accounts for a decrease of about 80% in the dry weight compared to the corresponding control.

Table 2: Variation in seedling shoot and root length (cm), fresh and dry weight (g plant^{-1}) of *Hordeum vulgare* and *Phalaris minor* as affected by different concentrations of *Arum cyreniacum* shoots crude powder (ACSCP), twenty-one days after sowing. Data are means of three replicates.

Concentration (%)	Shoot length (cm)	Root length (cm)	Fresh weight (g plant^{-1})	Dry weight (g plant^{-1})
	Hordeum vulgare			
0	23.333±1.528^{a}	25.333±1.528^{a}	40.25±0.203^{a}	16.307±0.471^{a}
10	15.733±3.10^{b}	12.666±1.528^{b}	33.261±0.211^{b}	12.857±751^{b}
20	16.666±2.52^{b}	15±2.65^{b}	27.076±0.05^{c}	9.993±0.325^{c}
40	14±1.00^{b}	11.333±1.155^{bc}	20.099±0.037^{d}	7.994±0.311^{c}
	Phalaris minor			
0	10±1.528^{a}	8.333±0.577^{a}	12.967±0.785^{a}	3.098±382^{a}
10	8±1.00^{b}	6.833±0.764^{b}	1.459±0.118^{b}	1.02±0.064^{b}
20	6±0.631^{bc}	1.666±0.577^{c}	0.409±0.015^{c}	0.254±0.115^{c}
40	0	0	0	0

Data are expressed as mean ±SD of 3 replicate. Within each row, means with different superscript (a, b, c or d) were significantly different at $p<0.05$. Where means superscripts with the same letters mean that there is no significant difference ($P>0.05$).

Photosynthetic pigments

The total content of the photosynthetic pigments of the recipient species upon applying ACSCP was presented in Table 3. The content in *p.minor* was reduced to 79.9% which may be ascribed to the decrease in both Chl.a and Chl.b. The reduction percentage in Chl.b, however was higher than that of Chl.a, it accounted to 82.68% in *p.minor* and 58.44% *H. vulgare* under concentration 20% compared to the corresponding control values. The percent reduction in carotenes, however, was less than that of chlorophylls a and b, it accounted to 79.01% and 31.24% in *p. minor, H. vulgare*, respectively. In the same way, the pigment content of seedlings was markedly reduced, and the percent reduction accounted to (79.9%, 47.8% in *p. minor, H. vulgare* respectively under 20% concentration.

Nutrient contents

Data recorded in Table 4 showed that the most pronounced effect was the decrease in K content of both treated plants. The percent decrease in K content of *H. vulgare* seedlings was 78.42% at 40% concentration while the percent decrease was 21.81% at 20% concentration of *P. minor* compared to the corresponding control values. Fe content decreased treatment in *H. vulgare* was 43.1% at 20% concentration and that the decrease was 62.5% at 20% concentration of *P. minor* compared to the corresponding control values.

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Table 3: Variation in the mean concentration of different pigment fractions (mg g fresh weight^{-1}) of *Hordeum vulgare* and *Phalaris minor* seedlings by *Arum cyreniacum* shoot crude powder (ACSCP), twenty-one days after sowing. Data are means of three replicates.

Conc. (%)	Chl."a"	Chl. "b"	Carotenoids	Chl. a/b ratio	Total pigments
	Hordeum vulgare				
0	17.385±0.124^{a}	8.7± 0.22^{a}	3.722±0.128^{a}	1.998± 0.041^{b}	29.807±0.242^{a}
10	12.956±0.336^{b}	5.584±0.139^{b}	3.227±0.154^{b}	2.320±0.042^{ab}	21.767±0.588^{b}
20	9.382±0.750^{c}	3.615±0.287^{c}	2.559±0.070^{c}	2.616±0.416^{a}	15.556±0.571^{c}
40	9.218±0.090^{c}	2.338± 0.098^{c}	1.243±0.121^{c}	3.165±0.269^{a}	12.799±0.034^{d}
	Phalaris minor				
0	11.653±0.112^{a}	4.873± 0.219^{a}	2.921±0.154^{a}	2.393±0.087^{b}	19.447±0.426^{a}
10	4.218±0.090^{b}	1.338± 0.098^{b}	1.243±0.121^{b}	3.165±0.269^{a}	6.799±0.034^{b}
20	2.44±0.0854^{c}	0.844±0.024^{c}	0.613±0.091^{c}	2.890±0.041^{a}	3.897±0.113^{c}
40	0	0	0	0	0

Data are expressed as mean ± SD of 3 replicate. Within each row, means with different superscript (a, b, c or d) were significantly different at $p<0.05$. Where means superscripts with the same significant difference ($P>0.05$).

Table 4: Variation in the concentrations of some nutrient elements of *Hordeum vulgare* and *Phalaris minor* seedlings as affected by *Arum cyreniacum* shoot crude powder (ACSCP), twenty one days after sowing and at maximum crude powder. Data are means of three replicates.

Conc. (%)	Na (ppm)	Cu (ppm)	K (ppm)	Ni (ppm)	Fe (ppm)
	Hordeum vulgare				
0	0.37± 0.010^{a}	3.8± 0.173^{b}	190± 3.00 ^{b}	0.612±0.011^{ab}	7.62± 0.361^{a}
10	0.3± 0.017^{b}	4.4± 0.173^{a}	170± 1.000^{b}	0.647±0.036^{a}	6± 0.265 ^{b}
20	0.29±0.010^{b}	4± 0.265^{ab}	210± 3.61^{a}	0.541±0.005^{c}	4.33± 0.017^{c}
40	0.28±0.027^{b}	3.8± 0.100^{b}	149± 1.73^{c}	0.565±0.036^{bc}	3.52± 0.027d
	Phalaris minor				
0	0.22±0.010^{b}	4.6± 0.100^{c}	220± 2.00^{a}	0.612±0.021^{b}	7.61± 0.121^{a}
10	0.29±0.010^{a}	15± 0.361^{a}	180± 2.65^{b}	0.835±0.003^{a}	3.58± 0.139^{b}
20	0.23±0.010^{b}	12.4± 0.173^{b}	48± 1.000^{c}	0.541±0.025^{c}	2.85± 0.010^{c}
40	0	0	0	0	0

Data are expressed as mean ± SD of 3 replicate. Within each row, means with different superscript (a, b, c or d) were significantly different at $p<0.05$. Where means superscripts with the same significant difference ($P>0.05$).

Discussion

Weeds are one of major constraints to plant production worldwide. Weeds affect crop growth and production that may be significantly reduced when weeds compete with them for light, water and minerals [13]. The reduction in seedlings radicle and coleoptile length in the present study may be attributed to the reduced rate of cell division and cell elongation due to the presence of allelochemicals in the aqueous extracts [14]. Several studies had shown that compounds of plant origin, such as allelochemicals, affect mitotic activity of growing roots [15,16]. Such an inhibitory effect on mitotic may directly decrease plant growth, and so mitotic activity can be used to evaluate root growth resulting from cell division of meristematic cells and cell expansion in the elongation zone of roots [17,18] reported that

DOI: https://doi.org/10.36811/ijpsh.2021.110032 **IJPSH: November-2021: Page No: 58-67**

the allelopathic effect of Euphorbia hierosolymitana reduced germination percentages, radicle and coleoptile growth of barley seedlings. Contrary finding on Wheat were also reported by [19] who found that the bark and leaf extract of Quercus glauca and Q. leucotricophora significantly reduced germination, plumule and radicle length of wheat. To the disturbance in the activities of peroxidase, alpha-amylase and acid phosphates [20]. This suggests with the aqueous extract of Deverra tortuosa moderately suppressed seed germination of a noxious weed; Medicago polymorpha under different concentrations [21]. Since this study was carried out under laboratory conditions, caution should be taken regarding the ecological implications of the data, because phytotoxicity of the different types of extracts is influenced by biotic and abiotic factors in soil. Although we expect similar trends in the growth response with recipient species other than P. minor, further research with different recipient plants in the field are desirable. [22] Showed that donor species; Artemisia monosperma, Peganum harmala and Silybum marianum extracts exhibited allelopathic action on the recipient species Chenopodium album as weed species and Triticum aestivum as crop species. The aqueous extract of all donor species has an inhibitory effect on the diurnal germination percentage of C. album seeds. Additionally, reserve mobilization, a process which usually takes place rapidly during early stages of seed germination seems to be delayed or decreased under allelopathy stress conditions [23]. Similar results were obtained by [24] who found a gradual increase of inhibition percentage of tomato (Lycopersicon esclentum) seed as a response to the higher concentration levels of Medicago sativa aqueous extract. [25] Found that seed germination of Raphanus sativus, Brassica campestris and Brassica oleracea was completely inhibited at >2% leaf extract of Parthenium hysterophorus. [26] Reported that the aqueous extracts prepared from the leaves and inflorescence of Artemisia monosperma negatively affected seed germination of some species of sandy habitat (e.g.Lasiurus scindicus, Pennisetum divisum, Scrophularia hypericifolia and Plantago boissieri) in a bioassay experiment. [27] reported that the highest germination percentage (GP) Portulaca oleraceae and Chenopodium album were occurred in control treatment and GP were decreased when the increase in extract concentrations of Crocus sativus and Peganum harmala. C. album was more sensitive to application of plant extracts than P. oleraceae. The same was obtained by [28] on the effect of Euphorbia helioscopia aqueous extract on seed germination of Triticum aestivum, Cicer arietinum, and Lens culinaris. [29] Found that the germination energy of Lolium westerwoldicum seeds was inhibited as response to the high concentration level of Taraxacum officinale extract. This could occur only when some allelochemicals present in the extract prevented growth of the embryo, or caused death. The extract of Parthenium hysterophorus induced a variety of chromosomal aberrations in dividing cells, which increased significantly with increasing concentrations and durations of exposure [30].

Hypophyllum tuberculatum aqueous extracts (HTAE) were tested on germination efficiency and growth parameters of *Lepidium sativum* and *Raphanus sativus* seeds. At the full-strength concentration (100%), the hypocotyl length (HL) was more sensitive than radicle length under HTAE. It was obvious that the allelopathic effect was prominent in *L. sativum* compared with *R. sativus* indicating the resistance of the latter to the allelochemicals extracted from HTAE [31,32].

In our study, significant reduction in the amount of chlorophyll a, chlorophyll b, total chlorophyll and carotenoids were recorded in response to the allelochemical stress of ACSCP. Reduction in Chl a, Chl b and total Chl were previously reported as a result of allelochemical stress[33-35] which could be attributed to the inhibition of chlorophyll biosynthesis (inhibition of supply orientation)

DOI: https://doi.org/10.36811/ijpsh.2021.110032 **IJPSH: November-2021: Page No: 58-67**

and/or the stimulation of chlorophyll degradation (stimulation of consumption orientation)[36,37]. [38] Reported reduction in chlorophyll content of *Vigna mungo* due to the allelochemicals present in leachate of black pepper which possibly target enzymes responsible for the conversion of porphyrin precursors. [39] Reported that the photosynthetic pigments of pepper seedlings were reduced by allelochemical stress. To go through with this, pepper-seedlings content of carotenoids was also decreased in response to allelochemicals. Carotenoids are pivotal accessory pigments playing major roles in photosynthesis by collecting light and transferring the excitation energy to the chlorophyll and by stabilizing proteins of the light-harvesting complex [40,41].

Allelopathic inhibition of mineral uptake was a domino effect results from alteration of cellular membrane functions in plant roots. Conclusive experiments have shown that specific allelochemicals (e.g. phenolic acids and flavonoids) inhibit mineral absorption by plant roots. The physiological mechanism of action of these allelochemicals involves the disruption of normal membrane functions in plant cells. These allelochemicals can depolarize the electrical potential difference across membranes, a primary driving force for active absorption of mineral ions [42]. In the present study, the concentration of almost studied nutrients in the two examined plant tissues was highly reduced. It could be concluded that the allelopathic compounds released from ACSCP significantly suppressed the uptake of the elements studied. Furthermore, [43] reported that the *Eucalyptus rostrata* water extract inhibits the uptake of N, P and K in *Vicia faba* and *Zea mays* seedlings.

Conclusion

Based on the results of this study, the species with the strong allelopathic potential, *Arum cyreniacum* must be examined for its selective action on other field conditions. Analysis of possible allelochemicals is also required. The isolation and characterization of growth inhibitors, which might be responsible for the strong allelopathic potential, are needed. There is possibility of using these allelochemicals for the discovery and development of environmental friendly herbicides to control weeds.

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