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Allellopathic effects of *Mesembryanthemum forsskalii* Hochst. ex Boiss. on seed germination and seedling growth of *Malva parviflora* L. and *Plantago ovata* Forssk.

Hediat M. H. Salama*, Mona S. Al Whibi

Botany and Microbiology Department, Faculty of Science, King Saud University, Riyadh, Saudi Arabia
*Corresponding author: Hediat M. H. Salama; E-mail: hoda.salama@hotmail.com

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ABSTRACT

The present study focused on the allelopathic effects of the aqueous and methanol extracts of *Mesembryanthemum forsskalii* Hochst. ex Boiss. on germination and seedling growth of *Malva parviflora* and *Plantago ovata. M. forsskaliii* was collected from Al-Jouf area, Saudia Arabia. The dried shoot system of *M. fosskalii* was used to prepare water and methanol extracts with different concentrations (25, 50, 75 and 100%) and distilled water as the control. The results showed that the aqueous and methanol extracts of *M. forsskalii* contained phenolic compounds and flavonoids that might be embroiled as allelochemical agents. Petri-dish trial showed that the two extracts at all concentrations reduced total germination percentage. Pot experiment indicated variations in seedlings germination and growth between *M. parviflora* and *P. ovata* in response to aqueous and methanol extracts of *M. forsskalii*. On growth stage the shoot and root lengths were decreased probably due to the allelopathic effects of *M. forsskalii.* The fresh and dry weights of shoot were inhibited with increase in concentration of aqueous and methanol extracts. In *M. parviflora* and *P. ovata* the leaf area was decreased under all concentrations. The chlorophyll a, b and carotenoids (pigments) were decreased in *M. parviflora* and *P. ovata* for all concentrations of extracts, but methanol extract increased chl. a only in *M. parviflora* compared to control. Flavonoids, saponins, tannins, carbohydrates, glycosides and phenolic are the allelochemical compounds released from the *M. forsskalii* into aqueous and methanol extracts which inhibited germination and growth of the studied plants.

Keywords: Allelopathy; *Mesembrynthemum forsskalii*; *Plantago ovata*; *Malva parviflora;* Chlorophyll; Germination; Growth.

1. INTRODUCTION

Allelochemicals liberated as residues, exudates and leaches by many plants from leaves, stems, roots, fruits and seeds reported to interfere with growth of other plants [1]. These chemical products mainly affect plants at seed emergence and seedling levels [2]. The effects of allelopathic are combined to many plants species and can be spotted at any level of biological organization [3, 4]. Plants extract that is not decomposed was thought to contain secondary compounds with allelochemical activity or phytotoxic which cause growth inhibition [5]. Allelochemicals are think to be a mutual action of

several secondary metabolites including terpenoids [6], juglone [7], flavonoids [8] and phenolic compounds [9]. Some researchers have pointed that the inhibitory materials implicated in allelopthy are terpenoids and phenolic material [10, 11].

Mesembryanthemum forsskalii belonging to the family Aizoaceae and is considered as an important medicinal plant. It is an erect, annual herb bearing fleshy terete to subterete linear leaves widely distributed in the all Middle East Countries and Saudi Arabia [12]. Due to its highest content of protein, fat and carbohydrates, in Kingdom of Saudi Arabia people utilize the seeds of *M. forsskalii* as food and mix the powder of seeds with butter and prepare a traditional recipe known as pakilla [13]. Furthermore, this desert plant also has noticeable medicinal importance on liver enzymes and lipid profiles of streptozotocin - induced diabetic in Wistar rats [14, 15]. The flavonoids, tannins, saponin, phenolics and anthocyanins are the most active chemical constituents of *M. forsskalii* [16]. *M. forsskalii* has anti inflammatory and cardio-protective effects, cytotoxic, antioxidant and antimutagenic [17]. The objective of the present study was to assess the allelopathic effects of *Mesembrynathemum forsskalii* on germination and seedling growth of *Malva parviflora* and *Plantago ovata* under lab and greenhouse conditions. *Malva parviflora* and *Plantago ovata* are widely distributed weeds with the economic agricultural crops and the present study try to control these plants by allelopathic effects.

2. MATERIAL AND METHODS

M. forsskalii was selected for this study because it recognized to produce allelochemicals. Shoot system of *M. forsskalii* was collected from Al-Jouf area in Saudi Arabia. Samples of *M. forsskalii* were washed completely with distilled water and dried in the open air for 14 days. Then dried samples were ground into fine powder and stored dry until used. The seeds *of Malva parviflora* and *Plantago ovata* were collected at the end of the growing season 2015.

2.1. Water extract preparation

10 g of air dried of *M. forsskalii* shoot was soaked in 100 ml distilled water for 48 hours at room temperature. The filter paper (Whatman No.1) was used to filter this extract. The filtered solutions were caught in a refrigerator until experiment start. The filtered solution (10% w/v) was diluted befittingly with distilled water to produce the final concentrations of 25, 50, 75 and 100%. The distilled water was used as the control treatment to assessment possibility of seeds germination.

2.2. Methanol extract preparation

10 g of air dried *M. forsskalii* shoot was extracted by 100 ml methanol in soxhlet apparatus for 24 hours [18]. After the rotary evaporator, the residue was dissolved in 3 ml methanol and completed to 100 ml by distilled water. It was prepared 25, 50 and 100% concentrations.

The phenolic content of *M. forsskalii* was estimated in the methanolic and aqueous extracts. The solvent systems H_2O: HOAc (47: 3) and BAW (4: 1: 5) were used to achieved paper chromatography on Whattman No. 1. HPLC were analyzed the samples [19].

2.3. Experiment of germination

5% sodium hypochlorite solution was used for sterilized the seeds of *M. parviflora* and *P. ovata* for 10 minutes, swill through with deionized water several times. In this experiment, 25 seeds were placed in each petri dish on filter paper, supplied with 15.0 ml of extracts or distilled water. The petri dishes were kept in a growth chamber at the controlled temperature (25 ± 3˚C). The petri dishes were closed by paper parafilms to prevent evaporation and pollution for 10 days. When the radical extended through the seed coat, the seeds germination was considered. The germinated seeds number was counted for each petri dish [20].

2.4. Seedling growth

Seeds of *M. parviflora* and *P. ovata* were germinated in pots. In each pot of 14 cm diameter and 18 cm height was filled with fertile loamy soil up to ¾ the height of the pot. Each pot was supplied with 15 ml of *M. forsskalii* extracts (25, 50, 75 and 100% of water, and methanol) and control was

added to every day, in three replicates. Plant growth in controlled temperature (25 ± 3˚C) illumination (dark/light cycle: 14/10 h) and 80% humidity into a greenhouse of Botany and Microbiology Department, Faculty of Science, King Saud University was conducted. The shoot and root lengths were measured after 35 days of growth. Also, fresh and dry weights of shoot and root were measured. The leaf surface area was measured using portable area meter Model Li-3000. The content of pigments chlorophyll a, b and carotenoids were accomplished based on method of Stirban [21].

2.5. Statistical analysis

Each treatment was conducted in a complete randomized with three replicates. The data were subjected to analysis with one way ANOVA test. The results were presented as mean ± SD (Standard Deviation). The significant differences between treatments means were separated using LSD test ($p<0.05$).

3. RESULTS AND DISCUSSION

Analysis of aqueous and methanol extract of *Mesembryanthemum forsskalii* showed that, three galloylglucose and four flavonol glycosides in different concentrations were present (Table 1). In this respect, flavonoids may leach from shoots into the soil solution and inhibit seed germination and root elongation [22]. The flavonoids also show antagonistic properties with plant hormones Indol Acetic Acid (IAA), metabolism and ion uptake by the plants [23].

3.1. Seed germination

The effect of *M. forsskalii* extracts on the seed germination percent of *M. parviflora* and *P. ovata* (Table 2). Compared to the control, the effect of water and methanol extracts at the low concentrations of 25 and 50% have slightly inhibition on the seed germination of *P. ovata.*

Table 1. Phenolic content of aqueous and methanolic extract of *M. forsskalii.*

Constituents	Aqueous extract µg/ml	Methanol extract µg/ml
1-O-galloyl-β-glucopyranose	3.4	5.2
1,6 di-O-galloyl-β- glucopyranose	5.1	4.9
1,3,6 tri-O-galloyl-β- glucopyranose	6.2	7.1
Quercetin 3-O-rutinoside	8.1	10.5
Quercetin 3-O-glucosylgalactoside	6.3	7.8
Quercetin 3-O-galactoside	8.3	11.6
Quercetin 3-O-glucoside	8.4	12.5

Table 2. Germination percentage of the investigated plants.

Treatment	*Malva parviflora*		*Plantago ovata*	
	Germination %	LSD 0.05	Germination %	LSD 0.05
Control	90±14.1		100 ± 0	
Water 100%	23±15	67(*)	74 ± 0.01	0.16 (*)
Water 75%	24 ± 16.1	66(*)	81 ± 0.01	0.19 (*)
Water 50%	26± 5	63.5(*)	84 ± 0.02	0.18 (*)
Water 25%	28 ± 15	62.5(*)	94 ± 0.02	0.06 (*)
Methanol 100%	10 ± 4	80(*)	73 ± 0.01	0.26 (*)
Methanol 50%	20 ± 28.28	70(*)	87 ± 0.01	0.02 (*)
Methanol 25%	40 ± 31.62	50(*)	93 ± 0.01	0.03 (*)

* The mean difference is significant at the 0.05 level.

Mean of three replications in duplicates ± Standard deviation.

Table 3. Length of shoot at start and after 35 days of treatment of the investigated plants.

Treatment	*Malva parviflora*				*Plantago ovata*			
	Length of shoot at the start of experiment (cm)	**LSD 0.05**	**Length of shoot after 35 days of experiment (cm)**	**LSD 0.05**	**Length of shoot at the start of experiment (cm)**	**LSD 0.05**	**Length of shoot after 35 days of experiment (cm)**	**LSD 0.05**
Control	26.3 ± 5.5		29.3 ± 5.5		10.3 ± 0.6		21.7 ± 1.2	
Water 100%	16. 7 ± 1.5	4.3 (*)	17. 7 ± 2.5	4.0(*)	9.3 ± 0.5	0.00	14.3 ± 0.3	7.3 (*)
Water 75%	21.7 ± 7.4	4.6 (*)	22.2 ± 6.9	7.1 (*)	10.3 ± 0.6	1.0 (*)	13 ± 2.7	8. 7(*)
Water 50%	22± 4.3	9.7 (*)	24 ± 4.4	9.6 (*)	10.3 ± 0.5	0.00	10.3 ± 0.6	11.6(*)
Water 25%	22.3 ± 4.7	4.0 (*)	25.3 ± 2.5	5.3 (*)	10.6 ± 0.3	0.30	13.7 ± 0.2	8.0 (*)
Methanol 100%	22.3 ± 2. 9	3.3 (*)	24± 2.6	4.7(*)	9 ± 0.1	1.3 (*)	11.3 ± 0.5	10.4 (*)
Methanol 50%	23± 1.7	4.0 (*)	24. 7 ± 2. 9	5.3 (*)	9 ± 0.1	1.2(*)	12 ± 0.1	9. 7(*)
Methanol 25%	24 ± 4.6	2.3	26.3 ± 3.2	3.0 (*)	10.3 ± 0.57	0.00	12.3 ± 0.7	9. 4(*)

* The mean difference is significant at the 0.05 level. Mean of three replications in duplicates ± Standard deviation.

Table 4. Length of root after 35 days of treatment of the investigated plants.

Treatment	*Malva parviflora*		*Plantago ovata*	
	Length of root after 35 days of experiment (cm)	**LSD 0.05**	**Length of root after 35 days of experiment (cm)**	**LSD 0.05**
Control	11.3 ± 1.5		16.333 ± 0.6	
Water 100%	10.7± 0.6	1.3	15.1 ± 0.5	1.2 (*)
Water 75%	12.6 ± 0.8	1.6 (*)	20.7 ± 2.3	4.3 (*)
Water 50%	13 ± 1	1.7 (*)	17.6 ± 0.5	1.3(*)
Water 25%	13.3± 0.6	0.7 (*)	21 ± 0	4.6 (*)
Methanol 100%	3.3± 0.5	8.0 (*)	14.7 ± 0.5	1.6 (*)
Methanol 50%	7.3 ± 3.2	4.0 (*)	12.4 ± 0.5	3.6 (*)
Methanol 25%	9.7± 2.8	1.6 (*)	13.3 ± 0.8	3.0 (*)

* The mean difference is significant at the 0.05 level. Mean of three replications in duplicates ± Standard deviation.

While, at high concentrations of 75 and 100% it is noticed the highly inhibitory effect. But, the seed germination of *M. parviflora* showed significant inhibition at all concentrations and the inhibition increase with increasing the concentration.

From these results, it is cleared that, the water and methanol extracts contained allelochemicals of growth inhibiting and their effects dependent on the concentration of *M. forsskalii* extract.

Seyed et al. concluded that the impact of different extract concentration of *Artemisia annua* on the germination percent is related to control and the least was related to the highest concentration 100% of extract [24]. The inhibitory effect of the extracts increased with increasing extract concentration [25, 26]. The present studies were confirmed with these studies and also with Salama and Al-Rabiah [27]. They concluded that, the effects of allelopathic can cause both stimulatory and suppressive effects at lower and higher concentrations respectively.

3.2. Shoot and root lengths

Table 3 shows that aqueous and methanol extracts had inhibition effect on shoot lengths in *M. parviflora* and *P. ovata* while higher concentrations (75 and 100%) induced greater inhibition after 35 days of processing. The aqueous extract revealed that the slightly inhibition of root lengths of *M. parviflora* and *P. ovata* at 100% and stimulated root lengths at 25, 50 and 75% (Table 4). However,

at all concentration the methanol extract exhibited significance inhibition of root lengths and the inhibitory effect increase with increasing extract concentration in *M. parviflora* and *P. ovata*. Mahmood et al. [26] gained similar results and concluded that methanolic extract significantly inhibited root and shoot growth of *Solanum melongena*. The effect of allelopathic contents of *M. forsskalii* has been imputing to the production of several active chemical constituents including flavonoids, saponins, tannins, carbohydrates, glycosides and phenolic compounds [16].

3.3. Fresh and dry weights

Both fresh and dry weights of shoot of *M. parviflora* had the highest inhibitory which affected by aqueous extract (1.45 and 0.15 g respectively), meanwhile, the highest weights of fresh and dry weights were recorded at low concentrations of aqueous and methanol extracts (2.61 and 3.28 g fresh wt.), (0.43 and 1.2 g dry wt.) respectively (Table 5). In *P. ovata* it was establish that there was low inhibitory effect of aqueous and methanol extracts on the fresh and dry weights of the shoot compared to control (Table 5). Generally, the highest concentrations induce the effect of allelopathic for *M. parviflora* and *P. ovata* at all extracts. The shoots recorded the maximum fresh and dry weights in untreated control. The present study was confirmed with Salama and Al-Rabiah [27] that studied allelopathic effects of *Citrullus colocynthis* on *Vicia faba* and *Hordeum vulgare.* In all extracts the fresh and dry weights in *M. parviflora* and *P. ovata* were reduced significantly. These results were confirmed with those obtained by Djanaguiraman et al. [28], who notice that seedling dry matter of rice, sorghum and blackgram significantly reduced by leaf leachate of *E. globules* and highest inhibition was observed in highest concentration. Aqueous eucalyptus extract decreased fresh and dry weights of three wheat cultivars [29].

3.4. Leaf surface area

Table 6 shows that the aqueous extract of *M. forsskalii* on the leaf area had significant inhibition at both low and high concentration (7.4 and 8.5 cm^2 respectively) compared to control for *M. parviflora*. However, the methanol extract of *M. forsskalii* showed slightly inhibition at high concentration and stimulation at low concentration for *M. parviflora*. The effect of all extracts of *M. forsskalii* on *P. ovata* showed significant inhibition of leaf surface area at all concentrations. These results are in an agreement with Salama and Al-Rabiah [27]. They concluded that any secondary compound with allelochemical activity can cause both inhibitory and stimulatory effects.

3.5. Chlorophyll content

The effect of aqueous and methanol extracts of *M. forsskalii* on the content of chlorophyll (Chl. a, b and carotenoids) were differ greatly on *M. parviflora and P. ovata*. Aqueous extract (25%) significantly inhibited chlorophyll a, b and carotenoids (0.06, 0.06 and 0.05 mg/g) on *M. parviflora* compared to control (Table 7). High concentrations of aqueous and methanol extracts (100%) catalyzed chlorophyll a, b and carotenoids (0.79, 0.99 and 0.46 mg/g) for aqueous extract and (0.97, 0.75 and 0.61 mg/g) for methanol extract. In *M. parviflora* aqueous extract with different concentrations (25, 50 and 75%) significantly inhibited Chl. a, b and carotenoids. Methanol extracts with different concentrations significantly stimulated chlorophyll a, b and carotenoids. The highest stimulatory effect on Chl. a, b and carotenoids were found in 100% methanol concentration being (0.97, 0.75 and 0.61 mg/g respectively) as shown in table 7. All the extracts effect of *M. forsskalii* on *P. ovata* showed significant inhibition of chl. a, b and carotenoids at all concentration and the inhibitory effect increase with increasing extract concentration. The present results are supported by the finding of Salama and Al-Rabiah [27], Corsato et al. [30] and Glieessman [31], they declared that the effect of allelopathic chemicals is a natural interference in which the plant produces substances and metabolites that may benefit or harm other plants when released. Also, these results were confirmed with Abdel-Fattah et al. [32] who found that the effects of allelopathic chemicals can cause both stimulatory and suppressive effects at higher and lower concentrations respectively.

Table 5. Fresh and dry weights of shoot after 35 days of treatment of the investigated plants.

Treatment	*Malva parviflora*.				*Plantago ovata*			
	Fresh wt. (g)	LSD 0.05	Dry wt. (g)	LSD 0.05	Fresh wt. (g)	LSD 0.05	Dry wt. (g)	LSD 0.05
Control	34.12 ± 0.98		7.43 ± 1.2		3.4 ± 0		0.21 ± 0.06	
Water 100%	1.45 ± 0.76	32.6 (*)	0.15 ± 0.04	7.18 (*)	2.3 ± 0	0.06 (*)	0.13 ± 0.06	0.07 (*)
Water 75%	1.55 ± 0.76	32.7 (*)	0.32± 0.04	7.08 (*)	2.6 ± 0	0.11 (*)	0.14 ± 0.06	0.13 (*)
Water 50%	1.68 ± 0.96	32.5 (*)	0.35 ± 0.02	7.11 (*)	2.8 ± 0.06	0.11 (*)	0.15 ± 0.06	0.10 (*)
Water 25%	2.61 ± 0.01	33.2 (*)	0.43 ± 0	7.08 (*)	2.9 ± 0.06	0.32 (*)	0.16 ± 0.06	0.04 (*)
Methanol 100%	2.26 ± 0.33	31.2 (*)	0.4 ± 0.1	6.82. (*)	2.19 ± 0.01	0.03 (*)	0.08 ± 0	0.12(*)
Methanol 50%	2.84± 0.29	31.8 (*)	0.58 ± 0.01	7.03 (*)	2.21 ± 0.01	0.03 (*)	0.10 ± 0	0.11(*)
Methanol 25%	3.28 ± 0.8	30.8 (*)	1.2 ± 0.2	6.23 (*)	2.22 ± 0.07	0.04(*)	0.11 ± 0.06	0.09 (*)

* The mean difference is significant at the 0.05 level. Mean of three replications in duplicates ± Standard deviation.

Table 6. Leaf area of the investigated plants.

Treatment	*Malva parviflora*		*Plantago ovata*	
	Leaf area (cm^2)	LSD 0.05	Leaf area (cm^2)	LSD 0.05
Control	12.2 ± 3.5		3.2 ± 0.08	
Water 100%	7.4 ± 0.03	4.8 (*)	0.8 ± 0.54	2.3 (*)
Water 75%	7.5 ± 0.31	4.7 (*)	1.0 ± 0.29	2.1 (*)
Water 50%	7.9 ± 0.45	4.3 (*)	2.5 ± 0.08	0.58 (*)
Water 25%	8.5 ± 0.01	4.7 (*)	2.7 ± 0.21	0.44 (*)
Methanol 100%	11.4 ± 0.99	0.83	1.0 ± 0.06	1.8 (*)
Methanol 50%	12.6 ± 3.87	0.40	1.2 ± 0.24	2.0 (*)
Methanol 25%	14.3 ± 2.71	2.0(*)	1.3 ± 0.05	2.0 (*)

* The mean difference is significant at the 0.05 level. Mean of three replications in duplicates ± Standard deviation.

Table 7. Chl.a, b and Carotenoids of the investigated plants.

Treatment	*Malva parviflora*						*Plantago ovata*					
	Chl.a mg/g	LSD 0.05	Chl.b mg/g	LSD 0.05	Carotenoid mg/g	LSD 0.05	Chl.a mg/g	LSD 0.05	Chl.b mg/g	LSD 0.05	Carotenoid mg/g	LSD 0.05
Control	0.76 ± 0.1		0.34 ± 0.1		0.26 ± 0.2		1.32 ± 0.0		1.45 ± 0.0		0.42 ± 0.0	
Water 100%	0.79 ± 0.01	0.70 (*)	0.99 ± 0.1	0.13 (*)	0.46 ± 0.1	0.20 (*)	0.54 ± 0.0	0.53 (*)	0.69 ± 0.0	0.6 (*)	0.2 ± 0.0	0.02 (*)
Water 75%	0.27 ± 0.01	0.48 (*)	0.32 ± 0.1	0.65 (*)	0.26 ± 0.1	0.00	0.6 ± 0.0	0.7 (*)	0.84 ± 0.0	0.4 (*)	0.3 ± 0.0	0.09 (*)
Water 50%	0.58± 0.02	0.18 (*)	0.31 ± 0.1	0.09 (*)	0.2 ± 0.1	0.06 (*)	0.9 ± 0.0	0.4 (*)	1.1 ± 0.0	0.3 (*)	0.4 ± 0.0	0.04 (*)
Water 25%	0.06± 0.1	1.2 (*)	0.06 ± 0.1	0.26 (*)	0.05 ± 0.1	0.21 (*)	1.0 ± 0.0	0.3 (*)	1.2 ± 0.0	0.2 (*)	0.4 ± 0.0	0.0.0 (*)
Methanol 100%	0.97 ± 0.1	0.23(*)	0.75 ± 0.01	0.07 (*)	0.61 ± 0.01	0.07 (*)	0.4 ± 0.0	0.7 (*)	0.5 ± 0.0	1.0 (*)	0.2 ± 0.0	0.05 (*)
Methanol 50%	0.83± 0.1	0.23 (*)	0.66 ± 0.1	0.01 (*)	0.35± 0.01	0.08 (*)	0.6± 0.0	0.7 (*)	0.7 ± 0.0	1.2 (*)	0.3 ± 0.0	0.2 (*)
Methanol 25%	0.79 ± 0.1	0.20 (*)	0.49 ± 0.1	0.14 (*)	0.28± 0.01	0.35 (*)	0.9 ± 0.0	0.4 (*)	0.8 ± 0.0	1.1 (*)	0.4 ± 0.0	0.13 (*)

* The mean difference is significant at the 0.05 level. Mean of three replications in duplicates ± Standard deviation.

4. CONCLUSION

The present study has shown that *Mesembryanthemum forsskalii* Hochst. ex Boiss. contained allelochemical compounds in their tissues and released these into aqueous and methanol solutions. The present research revealed that both aqueous and methanol extracts of shoot showed inhibitory effects on seed germination of *M. parviflora and P. ovata*. Aqueous and methanol extracts had inhibition effect on shoot lengths in *M. parviflora and P. ovata* and the inhibitory effect increase with increasing extract concentration. The fresh and dry weights of *M. parviflora and P. ovata* showed inhibition at all concentration of aqueous and methanol extracts. In *M. parviflora and P. ovata* the leaf area was decreased under all concentrations. The chlorophyll a, b and carotenoids were decreased in *M. parviflora and P. ovata* for all concentrations of extracts.

The effects of allelopathic contents of *M. forsskalii* has been imputing to the production of several active chemical constituents (flavonoids, saponins, tannins, carbohydrates, glycosides and phenolic compounds).

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AUTHORS' CONTRIBUTION

HMHS: Conception and design; Acquisition of data; Writing, review and revision of the manuscript; Administrative, technical or material support. HMHS and MSAW: Development of methodology; Analysis and interpretation of data. The final manuscript has been read and approved by both authors.

TRANSPARENCY DECLARATION

The authors declare that they have no conflict of interest.

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