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Allelopathic activity of leaves, stalks and roots of *Cymbopogon nardus*

P. Suwitchayanon* **and Hisashi Kato-Noguchi**

Department of Applied Biological Science, Faculty of Agriculture, Kagawa University, Miki, Kagawa 761-0795, Japan

Abstract

In this study, leaves, stalks and roots of *Cymbopogon nardus* were separately evaluated to determine the most active parts that contained the strong growth inhibitory activity. Each aqueous methanol extracts of *Cymbopogon nardus* were determined their allelopathic activity by using six test plant species; alfalfa (*Medicago sativa* L.), cress (*Lepidum sativum* L.), lettuce (*Lactuca sativa* L.), barnyard grass (*Echinochloa crus-galli* L.), Italian ryegrass (*Lolium moltiflorum* Lam.) and jungle rice (*Echinochloa colonum* (L.) P. Beauv.). Four extract concentrations (0.01, 0.03, 0.1 and 0.3 g dry weight equivalent extract/mL) were used for the bioassay. The results showed that these three extracts have inhibitory activity and the percent inhibition increased concentration dependently. However, the inhibitory activity of leaf and root extracts was more effective than stalk extract at 95% level of significance. Barnyard grass, Italian ryegrass and jungle rice were the most sensitive to the leaf, stalk and root extracts, respectively. The concentrations required for 50% growth inhibition of *C. nardus* leaf, stalk and root extracts on all test plants were 0.000-0.025, 0.009-0.077 and 0.003-0.023 g dry weight equivalent extract/mL, respectively. In addition, separation of these extracts through silica gel column indicated that root extract contained the most active fractions with strong growth inhibition. The present results suggest that *C. nardus* may have allelopathic compounds and the root extracts have the greatest inhibitory activity. Studies are in progress for the isolation and identification of allelopathic compounds in aqueous methanol extracts of *C. nardus* roots for the development of natural herbicides.

Key words: *Cymbopogon nardus,* Inhibitory activity, Aqueous methanol extract, Weed control

Introduction

Weed infestation in crop field results in a reduction in quality and quantity of crop productivity. Currently, synthetic herbicides have been considered to solve weed problems and prevented crop yield loss. However, the overuse of synthetic herbicide may affect the environment, human health and the increasing of herbicide resistance weeds (Owen and Zelaya, 2005; Hager and Refsell, 2008; Bhadoria, 2011). Hence, to overcome the disadvantages of herbicide applications, efforts to utilize natural plant products or natural eco-friendly chemicals are in demand.

Plant allelochemicals are defined as natural compounds that influence on the development of neighboring plants by releasing into the environment in several ways such as leaching, volatilization, root exudation and plant decomposition (Rice, 1984). The allelochemicals can be presented in every organ of plant parts including flowers, leaves, stems, roots and seeds (Rice, 1984; Fateh et al., 2012; Grisi et al., 2012). Naderi and Bijanzadeh (2012) identified the potential of allelopathic effects of leaf, stem and root extracts of ten Iranian rice cultivars on barnyard grass, which leaf extract exhibited the strongest growth inhibitory activity followed by root and stem extracts. Numerous researchers also reported that each plant part showed significant difference effects on the growth of test plant species (Dorning and Cipollini, 2006; Fateh et al., 2012; Grisi et al., 2012; Pirzad et al., 2012; Liu et al., 2003; Tabrizi and Yarnia, 2011). It has also been reported that the compounds in roots reach more easily to the surrounding plant roots than the compounds in leaves (Wu et al., 2009).

Cymbopogon nardus (L.) Rendle is a perennial grass that widely cultivated in Southeast Asia (Shasany et al., 2000; Nakahara et al., 2003). This plant is well known as mosquito repellent and also has several pharmacological properties (Simic et al., 2008; Nurhanani and Othman, 2010; Istianto

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*Corresponding Author

P. Suwitchayanon
Department of Applied Biological Science, Faculty of Agriculture, Kagawa University, Miki, Kagawa 761-0795, Japan

Email: s.prapaipit@gmail.com

and Emilda, 2011; Kongkaew et al., 2011; Silva et al., 2011; Sritabutra et al., 2011). For food productivity, Lonkar et al. (2013) analyzed the chemical constituents from leaves of six varieties of *C. flexuosus* for preparation as medicinal tea. However, there have been only a few studies of allelopathy in the genus of *Cymbopogon*. Zeng and Luo (1996) reported the effects of root exudates released by *C. citratus* that affected seedling growth of radish, rice and cucumber by decreasing seed germination, root length and seedling height. Additionally, the volatile compounds of *C. citratus* significantly inhibited seedling growth of corn and barnyard grass (Li et al., 2005).

Our previous studies confirmed that whole plants of *C. nardus* have strong inhibitory activity on common agricultural weeds such as barnyard grass, Italian ryegrass, jungle rice and timothy (Suwitchayanon et al., 2013). Therefore, in the present study, *C. nardus* was divided into three parts such as leaves, stalks and roots and investigated their allelopathic activities. The aqueous methanol extracts were determined their growth inhibitory activity on monocotyledonous and dicotyledonous species to develop as alternative weed management options.

Materials and Methods

Plant Materials

The whole plants of *Cymbopogon nardus* (L.) Rendle were collected from Chiang Mai province, Thailand in July 2012. Plants were washed several times to get rid of soil particles and separated into 3 parts; leaves, stalks and roots, then dried in oven at 70^{o}C and ground into powder. Dry powder was then vacuum sealed in a plastic bag and kept at 4^{o}C. Dicotyledonous species such as alfalfa (*Medicago sativa* L.), cress (*Lepidum sativum* L.) and lettuce (*Lactuca sativa* L.) were chosen because of their known seedling growth behavior. Monocotyledonous species such as barnyard grass (*Echinochloa crus-galli* L.), Italian ryegrass (*Lolium moltiflorum* Lam.) and jungle rice (*Echinochloa colonum* (L.) P. Beauv.) were chosen because there are common agricultural weeds.

Extraction

Leaf, stalk and root powder (100 g) was extracted separately with 1 L of 70% (v/v) aqueous methanol for two days. The extract of each plant powder was then filtered through one layer of filter paper (No. 2; Toyo Ltd., Japan), using a vacuum pump. The residue was extracted again with 1 L of cold methanol for one day and filtrated. The two filtrates of each part were combined and evaporated to dryness with a rotary evaporator at 40°C. Each crude extract was dissolved in cold methanol and subsequently used for the next experiments.

Bioassay

The bioassay was conducted with four concentrations (0.01, 0.03, 0.1 and 0.3 g dry weight equivalent extract/mL). An aliquot of the extract was added to a sheet of filter paper (No. 2) in 28 mm Petri dish. After the solvent evaporated, the filter paper was moistened with 0.6 mL of 0.05% (v/v) aqueous solution of polyoxyethylenesorbitan monolaurate (Tween 20; Nacalai, Kyoto, Japan), a surfactant that did not cause any toxic effects. Ten seeds of alfalfa, cress, lettuce or 10 germinated seeds of barnyard grass, Italian ryegrass or jungle rice were arranged on the filter paper in Petri dishes.

For germination, barnyard grass, Italian ryegrass and jungle rice were germinated by soaking in distilled water in Petri dish (9 cm diameter) and incubated in the darkness at 25°C for 72 h.

Control seeds were sown on the filter paper moistened with the aqueous solution of Tween 20 without the extract. The shoot and root lengths of those seedlings were measured at 48 h after incubation in the darkness at 25°C. Percent inhibition of seedling growth was calculated by reference to the length of control seedlings.

The bioassay was repeated three times with 10 plants for each determination. The inhibition percentage was calculated using the equation as follow: Inhibition (%) = [1-(treatment /control)] × 100. In addition, the concentrations required for 50% inhibition (I_{50}) of the test plant species in the assay were calculated from the regression equation of the concentration response curves.

Separation of the extracts

Leaf, stalk and root powder of *C. nardus* was extracted as described above. The extract was then concentrated at 40°C in vacuo to produce an aqueous residue. The aqueous residue was adjusted to pH 7.0 with 1 M phosphate buffer, and partitioned three times against an equal volume of ethyl acetate, and separated ethyl acetate and aqueous phase. The ethyl acetate fraction was carried out by drying over anhydrous Na_2SO_4 then filtrated and evaporated to dryness. The residue was chromatographed on 60 g of silica gel (60 Merck, 70–230 mesh) and eluted stepwise with *n*-hexane that contained increasing amount of ethyl acetate (10% per step, v/v; 150 mL per step) and methanol (300 mL). The inhibitory activity was determined by using cress seedlings.

Statistical Analysis

All experiments were carried out with triple replicated and repeat twice. The statistical data processing was analyzed by SPSS version 16.0 using one-way ANOVA and general linear model/ univariate. GraphPad Prism 6 was used to analyze the concentrations required for 50% inhibition.

Results

Effect of aqueous methanol extracts of leaves, stalks and roots of *C. nardus* on shoot growth

The inhibitory activity of *C. nardus* leaf, stalk and root extracts on shoot growth of six test plant species are shown in Figure 1. Three extracts showed different percent inhibition on test plant species. Threshold of growth inhibition for *C. nardus* leaf and root extracts was 0.03 g/mL while threshold of growth inhibition for stalk extract was 0.1 g/mL.

Leaf extract at the concentration of 0.03 g/mL completely inhibited shoot growth of lettuce (100%) and inhibited shoot growth of alfalfa, cress, Italian ryegrass, barnyard grass and jungle rice by 23.61, 28.74, 29.39, 39.30 and 51.28% of control, respectively.

Root extract at the concentration of 0.03 g/mL inhibited shoot growth of lettuce, alfalfa, cress, jungle rice, barnyard grass and Italian ryegrass by 4.21, 23.91, 25.29, 28.49, 30.39 and 31.91% of control, respectively.

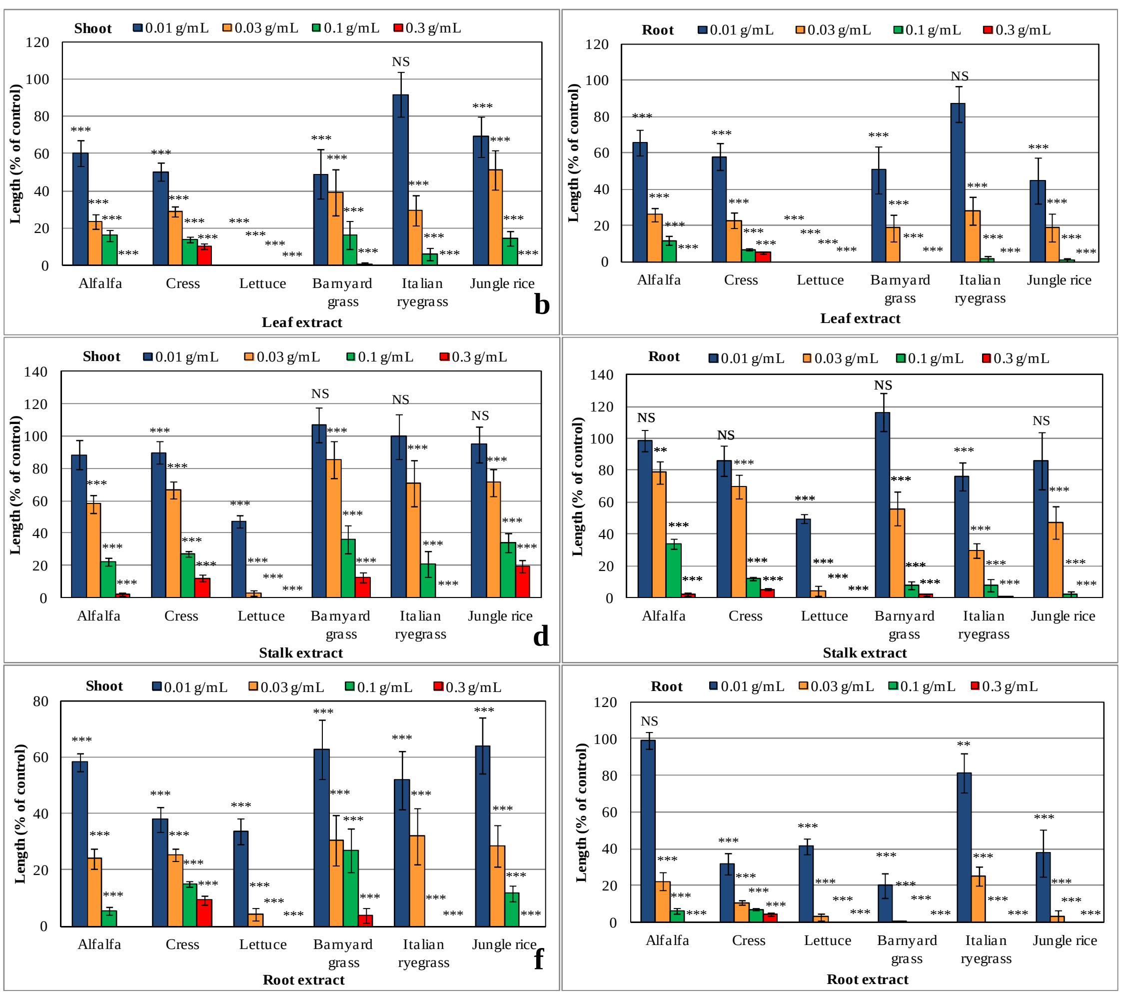

Figure 1. Effects of leaf (a, b), stalk (c, d) and root (e, f) extracts of *Cymbopogon nardus* on shoot and root growth of six test plant species. The bioassay was conducted with 0.01, 0.03, 0.1 and 0.3 g dry weight equivalent extract/mL. Means ± SE from three independent experiments with 10 seedlings for each determination are shown. *$P< 0.05$, **$P< 0.01$, ***$P< 0.001$ (Student's t-test).

Table 1. Effects of leaf, stalk and root extracts of *Cymbopogon nardus* on seedling growth of six test plant species.

Plant parts		Average % inhibition					
		Dicotyledonous species			Monocotyledonous species		
		Alfalfa	Cress	Lettuce	Barnyard grass	Italian ryegrass	Jungle rice
Leaf	Shoot	74.50 a	75.07 a	100.00 a	73.68 a	68.16 b	66.25 a
Stalk		57.36 b	51.44 b	87.50 c	39.85 b	52.12 c	44.98 b
Root		78.12 a	78.02 a	90.54 b	69.01 a	79.05 a	73.96 a
Leaf	Root	74.16 a	78.65 b	100.00 a	82.64 b	70.83 a	83.83 a
Stalk		46.64 c	57.01 c	86.54 b	54.57 c	71.50 a	66.31 b
Root		68.26 b	86.52 a	88.94 b	94.85 a	73.42 a	89.74 a

The bioassay was conducted with four concentrations; extracts obtained from 0.01, 0.03, 0.1 and 0.3 g dry weight of *C. nardus* per mL, and average % inhibition was calculated. Same letter in column is not significantly different at $P<0.05$.

Table 2. The concentration required for 50% inhibition on shoot and root growth of test plant species.

Plant parts	Test plant species	I_{50} (g dry weight equivalent extract/mL)	
		Shoot	Root
Leaf	Alfalfa	0.013	0.015
	Cress	0.009	0.012
	Lettuce	0.000	0.000
	Barnyard grass	0.012	0.010
	Italian ryegrass	0.022	0.021
	Jungle rice	0.025	0.009
Stalk	Alfalfa	0.039	0.066
	Cress	0.050	0.042
	Lettuce	0.010	0.010
	Barnyard grass	0.077	0.030
	Italian ryegrass	0.049	0.019
	Jungle rice	0.066	0.028
Root	Alfalfa	0.013	0.023
	Cress	0.004	0.004
	Lettuce	0.007	0.009
	Barnyard grass	0.016	0.007
	Italian ryegrass	0.012	0.019
	Jungle rice	0.015	0.008

The values were determined by a logistic regression analysis after bioassays

Stalk extract at the concentration of 0.1 g/mL completely inhibited shoot growth of lettuce seedling (100%) and inhibited shoot growth of Italian ryegrass, alfalfa, cress, jungle rice and barnyard grass by 20.76, 22.38, 26.92, 33.95 and 36.02% of control, respectively.

Comparison of the average percent inhibition of these three extracts is shown in Table 1. Leaf and root extracts have greater percent inhibition than stalk extract and demonstrated significantly different from stalk extract ($P<0.05$).

The concentration required for 50% inhibition (I_{50}) is shown in Table 2. Leaf and root extracts exhibited I_{50} at 0.00-0.025 and 0.004-0.016 g/mL, which were lower that stalk extract at 0.010-0.077 g/mL. For dicotyledonous species, all plant extracts were strongly inhibited lettuce and cress shoots. For monocotyledonous species, barnyard grass shoots were the most sensitive to leaf extract and Italian ryegrass shoots were the most sensitive to stalk and root extracts.

Effect of aqueous methanol extracts of leaves, stalks and roots of *C. nardus* on root growth

The inhibitory activity of *C. nardus* leaf, stalk and root extracts on root growth of six test plant species are shown in Figure 1. Leaf extract at the concentration of 0.03 g/mL completely inhibited root growth of lettuce (100%) and inhibited root growth of barnyard grass, jungle rice, cress, alfalfa and Italian ryegrass by 18.69, 18.80, 22.71, 26.12 and 28.18% of control, respectively.

Root extract at the concentration of 0.03 g/mL inhibited root growth of barnyard grass, lettuce, jungle rice, cress, alfalfa and Italian ryegrass by 0.59, 2.93, 3.21, 10.54, 22.21 and 25.00% of control, respectively.

In contrast with leaf and root extracts, stalk extract required the concentration of 0.1 g/mL for completely inhibited root growth of lettuce seedling (100%) and inhibited root growth of jungle rice, barnyard grass, Italian ryegrass, cress and alfalfa by 1.83, 7.57, 7.85, 12.20 and 34.03% of control, respectively.

Table 1 shows that the inhibition of plant part extracts on root growth of test plant species were also corresponding to their shoot growth (P<0.05).

Leaf and root extracts exhibited I_{50} at 0.00-0.021 and 0.004-0.023 g/mL, which were lower than stalk extract at 0.010-0.066 g/mL (Table 2). For dicotyledonous species, lettuce and cress roots were sensitive to all plant extracts than their shoot. For monocotyledonous species, jungle rice roots were the most sensitive to leaf extracts while Italian ryegrass and barnyard grass roots were the most sensitive to stalk and root extracts, respectively.

Separation of the extracts

Several active fractions separated by silica gel column were found in the root, leaf and stalk extracts (Figure 2). Six fractions eluted with 30 (F2), 40 (F3), 50 (F4), 60 (F5), 70 (F6) and 80% ethyl acetate (F7) in *n*-hexane separated from root extract inhibited shoot growth of cress seedling by 36.32, 22.11, 45.79, 28.42, 15.79 and 34.74% of control, respectively. Those fractions also inhibited root growth by 29.80, 7.45, 46.05, 18.28, 6.09 and 23.70% of control, respectively.

Three fractions eluted with 60 (F5), 70% ethyl acetate (F6) in *n*-hexane and methanol (F9) separated from leaf extract inhibited shoot growth by 33.71, 42.13 and 47.19%, respectively and inhibited root growth by 36.88, 36.88 and 35.46% of control, respectively.

In contrast, only one active fraction, which was eluted with 70% ethyl acetate in *n*-hexane (F6), was detected in stalk extract. The activity of the fraction on cress shoots and roots was 27.57 and 24.57% of control, respectively.

Discussion

Aqueous methanol extracts of *C. nardus* leaves, stalks and roots exhibited different growth inhibitory activity which were depending on test plant species such as monocotyledonous species (barnyard grass, Italian ryegrass and jungle rice) and dicotyledonous species (alfalfa, cress and lettuce), and also the extract concentrations. The inhibition increased with increasing extract concentrations (Figure 1). Islam and Noguchi (2013) also indicated that the growth restriction of cress and Italian ryegrass were more clearly observed with increasing concentration of allelopathic compounds.

a

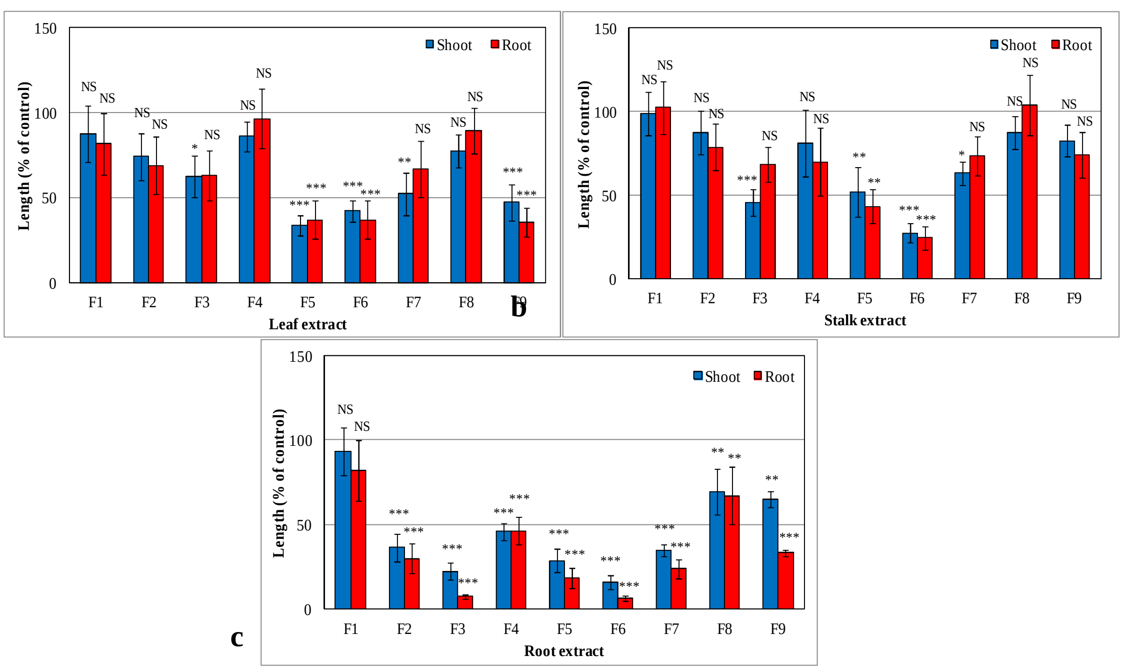

Figure 2. Effects of fractions separated by a silica gel column on seedling growth of cress. The bioassay of *Cymbopogon nardus* leaf (a), stalk (b) and root (c) extracts was conducted at the concentration of 0.3 g dry weight equivalent extract/mL. Means ± SE from three independent experiments with 10 seedlings for each determination are shown. *P< 0.05, **P< 0.01, ***P< 0.001 (Student's t-test).

The threshold concentration for growth inhibition on the test plants was lower in leaf and root extracts than stalk extracts ($P<0.05$) (Table 2). In addition, effectiveness of the extracts to test plant roots was greater than that to their shoots which correspond to the reported by many researchers (Olofsdotter et al., 2002; Pukclai et al., 2010; Zhang and Fu, 2010; Hussain and Reigosa, 2011; Esmaeili et al., 2012). It may be possible reason that roots easily contact and absorb the compound in the medium and soil (Salam and Noguchi, 2010).

The present results show that leaf and root extracts have greater inhibitory activity on test plant species than stalk extract, and also required lower concentration for 50% growth inhibition than stalk extract (Table 2). It was reported that leaves and roots were the main source of allelopathic compounds (Rice, 1984; Fateh et al., 2012). Dicotyledonous species were more sensitive to all plant extracts than monocotyledonous species (Table 1), which the inhibitory activity of the allelopathic substances was species specific and concentration dependent (Barnes and Putnam, 1987; Kruse et al., 2000).

Separation of *C. nardus* root extract through silica gel column showed the most active fractions and stronger growth inhibitory on cress seedlings than leaf and stalk extracts (Figure 2). Additionally, these three extracts had the same two active fractions, F5 and F6, however those fractions of each extract exhibited different level of growth inhibitory activity. It may be due to the differences in concentrations of allelopathic substances or variation of chemical composition between plant parts (Wu et al., 2009; Grisi et al., 2012; Sarkar et al., 2012).

Conclusions

Aqueous methanol extracts of leaves, stalks and roots of *C. nardus* exhibited the growth inhibitory activity especially on common weeds in agriculture fields such as barnyard grass, Italian ryegrass and jungle rice. Leaf and root extracts demonstrated strong growth inhibition. This study suggests that the growth inhibitory activity of each extract may be due to the allelopathic substances in *C. nardus*. The isolation and identification of allelopathic substances in aqueous methanol extracts of *C. nardus* roots are in progress with the purpose for development of natural herbicide for controlling weed control purpose.

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