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Research Article

Bioactivity of Powder and Extracts from Garlic, *Allium sativum* L. (Alliaceae) and Spring Onion, *Allium fistulosum* L. (Alliaceae) against *Callosobruchus maculatus* F. (Coleoptera: Bruchidae) on Cowpea, *Vigna unguiculata* (L.) Walp (Leguminosae) Seeds

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Laboratory bioassays were conducted to investigate the bioactivity of powders, extracts, and essential oils from *Allium sativum* L. (Alliaceae) and *A. fistulosum* L. (Liliaceae) against adults, eggs, and larvae of *Callosobruchus maculatus* F. (Coleoptera: Bruchidae). On the basis of 48 hr median lethal toxicity (LC_{50}), test plant powders and extracts from *A. sativum* were more toxic to *C. maculatus* adults than those from *A. fistulosum*. The 48 hr LC_{50} values for the powder against the test insect species were 9.66 g/kg and 26.29 g/kg for *A. sativum* and *A. fistulosum*, respectively. Also the 48 hr LC_{50} values obtained show that aqueous extracts of the test plant species, 0.11 g/L (*A. sativum*) and 0.411 g/L (*A. fistulosum*) were more toxic to *C. maculatus* than the corresponding ethanol extracts. There was no significant difference in the toxicity of vapours from the two test plant species against *C. maculatus*, although *A. sativum* gave lower values. The study shows that *A. sativum* and *A. fistulosum* have potentials for protecting stored cowpea from damage by *C. maculatus*.

1. Introduction

Grain storage has often resulted in quantitative and qualitative losses due to physical, chemical, and most importantly biological factors such as pests which may be birds, rodents, fungi, or insects [1–3]. The most important among storage pests are insects because apart from their direct damage they create conditions that allow secondary infestation by rot organisms mainly fungi [1, 4].

Once infestation is established pest insects cause gradual and progressive damage leading to losses in weight, nutritional, organoleptic, and aesthetic quality of stored grains. Osuji [1] listed 40 insects affecting stored grains, the most important among which is the cowpea weevil, *Callosobruchus maculatus* F. (Coleoptera; Bruchidae) responsible for up to 100% infestation of cowpea, *Vigna unguiculata* (L.) Walp (Leguminosae) during storage [1, 3, 5]. These observations justify the control of insect pests like *C. maculatus* in order to reduce losses in stored cowpea.

Several methods are used in controlling insects in stored grains, including physical (smoking, sun-drying, heating), cultural, biological (male insect sterilization, natural enemies, resistant grain varieties), and chemical (synthetic and natural products) methods. The most common and widely used is the chemical method involving mainly the use of synthetic insecticides.

Several workers have reported the successful wide scale use of synthetic organic insecticides, commencing with the organochlorines in the middle 1940s, followed by the later use of organophosphates, carbamates, pyrethroids, avermectins, and others. Insecticides most commonly used to protect stored grains from insect pests include aluminium phosphide, lindane, methyl bromide, ethylene dibromide, edifenphos, pirimiphos methyl, permethrin, malathion, sumithion, chlorpyrifos methyl, chlorpyrifos, propoxur, fenithrothion, dichlorvos, bromophos, fenvalerate, bioresmethrin, phenothrin, and deltamethrin [3].

The observed overreliance on insecticides was mainly, due to their initial quick action, ease of use and general efficiency in reducing pest populations and damage. However, there are limitations to their use mainly the deleterious side-effects to nontarget species including humans and the development of resistant strains of pests [6, 7]. In addition to these limitations, there is also the problem of high cost of synthetic insecticides, which is a limiting factor particularly to the largely peasant farmers of Africa including Nigeria.

Due to the foregoing reasons, there has been a need to search for new insecticides with novel mechanism of action. In this regard, many scientists have reasoned that it is advantageous to investigate natural products as a source of degradable insecticides that may turn out to be safer to humans and the rest of the environment than the synthetics.

In the present study, garlic, *Allium sativum* (Alliaceae) and Spring onion, *A. fistulosum* (Alliaceae) were screened for their bioactivity against *C. maculatus*. Members of the genus *Allium* have been known to demonstrate repellent and insecticidal properties against medically important insect pest species [8] and a few workers including Stoll [9] and Oparaeke et al. [10] have reported their potency against other insects. However, there is a dearth of studies on the bioactivity of extracts and volatile oils from these plant species against *C. maculatus*, especially their eggs and larvae. Understanding the toxicity of compounds to adults and immature stages is very important as it would indicate the appropriate time to apply them for adequate control of the insect pests. The present study would therefore provide the needed information on the toxicity of the extracts and volatile essential oils from *A. sativum* and *A. fistulousm,* respectively, against adult, larva, and egg of *C. maculatus*.

2. Materials and Methods

2.1. Test Plant Materials. The cloves of garlic *A. sativum* and leaves of Spring Onion *A. fistulosum* obtained from Iyana Iba market, Lagos, were the test plant materials used.

Test plant materials were used against test insect species in four formulations, namely, powder, aqueous and ethanol extract of powders, and essential oils prepared as described below. To prepare the powder, plant parts were first dried slowly to constant weight in a wooden cabinet (1.0 m × 0.5 m × 1.0 m) fitted with 100 watts bulb, which provided an average temperature of about 42°C for 7–14 days before pulverization in a Binatone blender (model No. BLG 400). The powders were passed through sieve of 0.1 mm mesh size to standardize particles size.

Aqueous and ethanol extract were each prepared from the powder. In each case, 500 g of plant powder was steeped in 1 L of water or ethanol that served as solvent, for 24 hrs. The mixture was then passed through Whatman No. 1 filter paper (15 cm diameter). The filtrate in each case was stored in a labelled Kilner jar while the residue was reextracted with water or ethanol, respectively, and all filtrates combined for each treatment. Each of the combined filtrates was then dried over a water bath at 50°C temperature and the resultant residue used as crude active ingredient. Volatile essential oil was extracted from 500 g of pulverised *A. sativum*, or *A. fistulosum* by hydrodistillation for 7–8 hrs in a Clavenger apparatus [11], collecting the volatile oil over hexane, which was removed by passing it over anhydrous sodium sulphate. Each of the essential oils was stored in glass vials kept in refrigerator at 4°C to reduce evaporative loss until when needed for bioassays.

2.2. Callosobruchus Maculatus Culture. Cowpea weevil, *C. maculatus* (F.) starter cultures obtained from the insectary of Nigerian Stored Product Research Institute (NSPRI), Abule-Oja, Lagos, where they have been held in cultures for decades unexposed to insecticide were used. Fresh experimental cultures were prepared from the original stocks and maintained at 30 ± 1°C temperature and 70 ± 4% relative humidity as described by Denloye et al. [12]. *Callosobruchus* was maintained on cowpea seeds. The grains were disinfested by picking those with damage holes and heating in the oven at 50°C for five hours. Disinfested grains were measured into clean 1 L Kilner jars with screw caps. Each jar contained 500 g of cowpea into which seven 0-1-d old adult *C. maculatus* (2 ♂, 5 ♀) were introduced. All adult *C. maculatus* were removed from the culture after seven days for oviposition to take place. Fresh cultures were made from this for subsequent tests.

3. Bioassays

3.1. Acute Toxicity of Plant Powders. Twenty active 0–3-day-old *C. maculatus* (mixed sexes) were exposed to disinfested cowpea grains admixed with powdered plant material at concentrations ranging between 5.0 g/kg and 320 g/kg or without plant material as control in disposable plastic cups covered with muslin.

3.2. Acute Toxicity of Aqueous and Ethanol Extracts. Similar sets of experiments as described above were carried out, but this time grains were treated by dipping them for approximately 30 secs in different concentrations (0.5–16 g/L) of each plant extract.

3.3. Fumigant Toxicity of Volatile Essential Oils

3.3.1. Adults. Fumigation bioassays were carried out in 1 L airtight Kilner jars using the method of Don Pedro [13, 14]. In this procedure, a 7 cm-diameter Whatmann No. 1 filter paper was always impregnated uniformly with a test essential oil at predetermined concentrations, and quickly hung with a thread in the fumigation chamber already holding 20 adult test insects. The chamber was then sealed with the cap, screwing the ring holding a glass lid tightly on to a rubber washer covered with aluminium foil to prevent reaction with essential oil. The cap remained tightly screwed to ensure fumigation in the airtight chamber for 24 hrs. In controls, insects were left in airtight sealed chambers without oil on the filter paper. There were four replicates per treatment. After the 24 hr fumigation the chambers were opened and the insects that were still alive transferred into recovery

Table 1: Acute (48 h) toxicity of test plant materials against *Callosobruchus maculatus.*

Formulation	Test plant species	LC_{50} 95% Confidence Limits	LC_{95} 95% Confidence Limits	Regression equation	DF	Slope (±SE)
Powder (g/kg)	*A. sativum*	9.661 (7.957–11.691)	70.143 (50.983–96.317)	$Y = -1.888 + 1.916x$	4	1.916 ± 0.031
	A. fistulosum	26.293 (20.485–33.632)	501.742 (293.804–854.42)	$Y = -1.829 + 1.288x$	4	1.288 ± 0.018
Aqueous extracts (g/l)	*A. sativum*	0.110 (0.087–0.137)	1.30 (0.80–2.17)	$Y = -1.475 + 1.583x$	3	1.538 ± 0.03
	A. fistulosum	0.411 (0.314–0.510)	4.017 (2.788–6.659)	$Y = 0.643 + 1.667x$	5	1.667 ± 0.035
Ethanol extracts (g/l)	*A. sativum*	0.219 (0.181–0.261)	1.297 (0.959–1.803)	$Y = 1.409 + 2.134x$	3	2.134 ± 0.046
	A. fistulosum	0.863 (0.687–1.072)	12.955 (7.624–28.913)	$Y = 0.089 + 1.403x$	3	1.403 ± 0.027

DF: Degree of Freedom; SE: Standard Error.

chambers. Mortality counts were taken in the recovery chambers every 24 hrs for seven days.

3.3.2. Eggs. Fumigation of *C. maculatus* eggs on cowpea was carried out in 1 L airtight Kilner jar using 0.5 mL of *A. sativum* or *A. fistulosum* oil, respectively. Twenty seeds bearing one egg each were assayed against each of the test oils and replicated four times. A control, also replicated four times, was set up similarly but the filter paper had no oil. The egg bearing cowpeas were transferred after 24 hours to ventilated plastic cups and later inspected for hatched (or unhatched) eggs under a stereomicroscope with X 8 objective after 12 days.

3.3.3. Larvae. Another similar experiment was set up with the arrangement described above using 6–8-day-old hatched eggs (i.e, 1-2-day-old larvae) since eggs hatch into larvae after 6 days of incubation. A batch of 20 cowpea seeds, each of which had one 6–8-day-old eggs were placed in fumigation chamber having 7 cm diameter filter paper impregnated with various concentrations of test oils. After 24 hours of fumigation, the cowpea seeds were transferred into ventilated plastic cups and left for 21 days. Each treatment and control was replicated four times. Mortality was assessed by dissecting each cowpea seeds to recover dead (or living) larvae.

4. Persistence of Test Plant Materials

4.1. Extracts. Forty undamaged cowpea grains were treated by dipping for approximately 30 secs in predetermined concentrations (0.5 to 8.0 g/L) of aqueous extracts of either *A. sativum* or *A. fistulosum*, and allowed to drain on filter paper for 5 minutes before transferring into bioassay containers. Several sets of treated seeds and two controls were prepared. For each set of treated seeds and controls, bioassays were started off by introducing 10 adult *C. maculatus* aged 0–3 days at preset times expressed as Hours After Treatment (HAT), namely, 0 (immediately after treatment), 12, 24, 96, 168, and 336 HAT. Each treatment and control was replicated four times. Each set of experiments was assessed by taking mortality of test insects every 12 hours for 336 hours.

4.2. Essential Oils. Similar experiments were carried out using concentrations (0.8 mL/L to 12.80 mL/L) of essential oil of *A. sativum* and *A. fistulosum,* respectively, instead of aqueous extracts.

4.3. Assessment of Mortality. In all bioassays insects were counted as dead when they failed to move any part of their body after prodding with fine brush bristle.

4.4. Data Analyses. Quantal responses (mortality) of *C. maculatus* were subjected to probit analysis [15] using computer software after correcting for mortality with Abbot formula [16]. From these analyses, LC_{50} (the concentration at which 50% of test insects died at a given time) and LC_{95} values of test plant materials were computed.

5. Results

5.1. Acute Toxicity of Test Plant Powders to C. maculatus. The 48 hr LC_{50} values of *A. sativum* (9.66 g/kg) and *A. fistulosum* (26.29 g/kg) and their corresponding LC_{95} values against *C. maculatus* are shown in Table 1. Powdered *A. sativum* was significantly more toxic to the test insect species than *A. fistulosum* (no overlap in 95% confidence limits).

5.2. Acute Toxicity of Test Plant Extracts to C. maculatus. The aqueous extracts were more toxic to *C. maculatus* than the ethanol extracts. Probit analysis show that the 48 h LC_{50} values of the *A. sativum* aqueous extract was 0.11 g/l, a value lower than that of *A. fistulosum* (0.41 g/l). For the ethanol extracts, the *A. sativum* gave LC_{50} value of 0.22 g/l which is 4X lower than the corresponding value for *A. fistulosum* shown in the Toxicity Factor column (Table 1).

5.3. Fumigant Toxicity of Test Essential Oils to C. maculatus Adult, Eggs, and Larvae. There was no significant difference in the toxicity of *A. sativum* essential oil when compared with that of *A. fistulosum* (no overlap in 95% confidence limits) although *A. sativum* gave lower LC_{50} and LC_{95} values relative to *A. fistulosum* essential oil (Table 2) against both the adults and the eggs, respectively. The essential oils of *A. sativum* and *A. fistulosum* resulted in mortality of the larvae of *C. maculatus* in the cowpea grains, though below 20%.

Table 2: Fumigant toxicity of test essential oils to *C. maculatus* adults and eggs.

Test insect species	Test plant species	LC_{50} (95% Confidence limits)	LC_{95} (95% Confidence limits)	Regression equation	DF	Slope (±SE)
Adults	*A. sativum*	15.46 (12.44–19.153)	157.122 (104.97–235.058)	$Y = -1.948 + 1.638x$	3	1.638 ± 0.024
	A. fistulosum	23.144 (18.403–29.059)	363.125 (205.718–643.59)	$Y = -1.883 + 1.38x$	3	1.38 ± 0.021
Eggs	*A. sativum*	14.536 (11.826–17.953)	142.789 (79.183–262.334)	$Y = -1.933 + 1.663x$	3	1.663 ± 0.032
	A. fistulosum	20.844 (15.589–28.232)	335.986 (137.429–858.69)	$Y = -1.802 + 1.367x$	3	1.367 ± 0.031

DF: Degree of Freedom; SE: Standard Error.

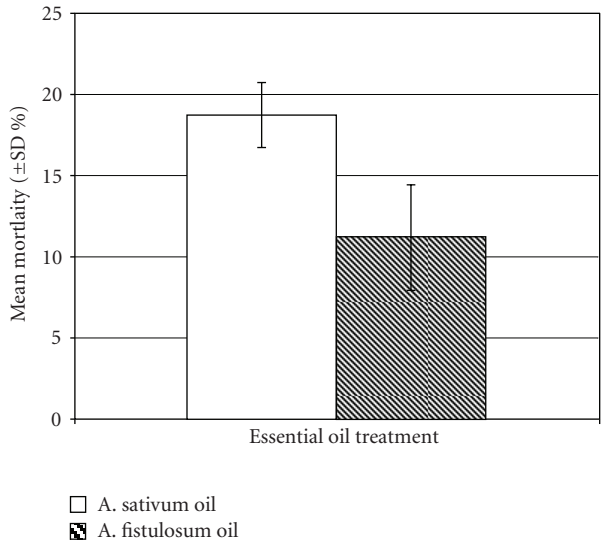

Figure 1: Fumigant toxicity of essential oil of Allium spp against *C. maculatus* larvae.

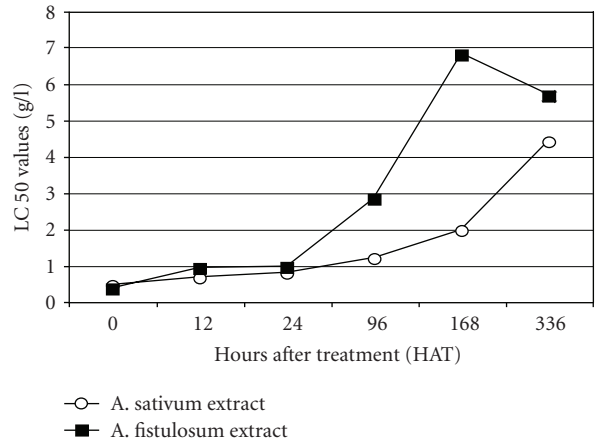

Figure 2: Persistence of test plant extracts against *C. maculatus* adult.

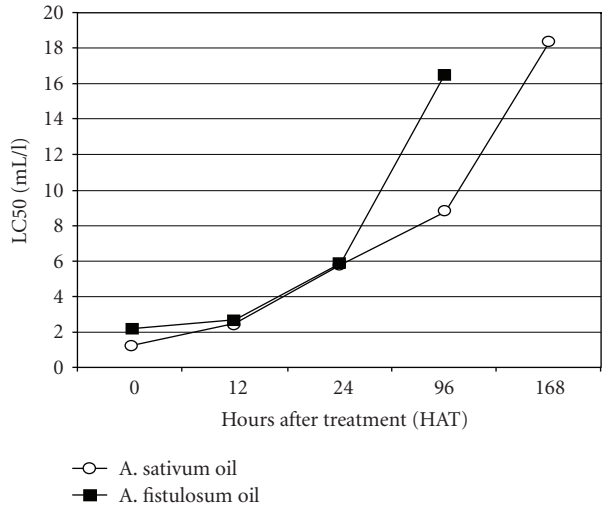

Figure 3: Persistence of test plant essential oils against *C. maculatus* adult.

A. sativum resulted in a higher number of dead larvae than *A. fistulosum* oil (Figure 1).

6. Persistence of Plant Extracts and Oils for Bioactivity against *C. maculatus*

6.1. Extracts. The persistence of the toxicity of aqueous extracts of both test plant species is shown in Figure 2. The computed LC_{50} values for the two test extracts increased slightly by 12 hrs and was maintained up to 24 hrs. The ethanol extract of *A. fistulosum* was less persistent than that of *A. sativum* (Figure 2).

6.2. Essential Oils. The potency of the oils from the two test plant species remained only for 12 hrs, after which it was lost rapidly. The potency of *A. fistulosum* oil was completely lost by 96 HAT (Figure 3).

7. Discussion

The results demonstrate that although *A. sativum* and *A. fistulosum* are of the same genus, they showed different potencies against the adults, eggs, and larvae of *C. maculates,* respectively. The powder of *A. sativum* gave high toxicity against *C. maculatus* adults with LC_{50} values of 9.66 g/Kg. This value show that powdered *A. sativum* was equally toxic to *C. maculatus* as Citrus species in studies carried out by Don-Pedro [17] and Kellouche and Soltan [18].

The extracts of *A. sativum* were more toxic to *C. maculatus* than those of *A. fistulosum* in this study. This may be because the active principles responsible for the activity of

the test extracts were present in higher quantities in the *A. sativum* than the *A. fistulosum*. In addition, *A.sativum* may contain other compounds not contained in *A. fistulosum*. Our study shows that the aqueous extracts were toxic to *C. maculatus*, thus reinforcing earlier observations that members of the genus *Allium* are potent against insects. Denloye and Makanjuola [19] and Denloye et al. [8, 20] have reported the insecticidal potency of the aqueous extracts of *A. sativum* against *Sitophilus zeamais* and *Anopheles* species. Our results from the present study agree with these earlier reports.

The solvent used in extracting plant materials for insecticidal potency is highly important as our present study shows. Ethanol extracts were less toxic than the aqueous extracts. This agrees with earlier reports that aqueous extracts of garlic *A. sativum* were more toxic to *S. zeamais* than the methanolic extract [20]. This could be because the active principles in the test plant materials are more soluble in water. Grieve [21] stated that the higher efficacy of aqueous extracts over that of ethanol is due to the fact that alkyl compounds present in the Alliacea family are readily obtained by distillation with water. Our results in the present study show that the effectiveness of a natural plant extracts increase with decreasing polarity of the solvent used for extraction in agreement with earlier reports by Denloye et al. [20] and Ojewole et al. [22].

The ovicidal action of the essential oils from test plant species have been demonstrated in this study. This indicates that *A. sativum* and *A. fistulosum,* like other plants with essential oils having ovicidal effects [13, 14, 23], may be exploited for the prevention and control of *C. maculatus* infestation of stored cowpea. Overall, the results obtained from this study portend greater usefulness for *A. sativum* as a source of bioactive formulations capable of protecting stored cowpea from infestation by *C. maculatus.*

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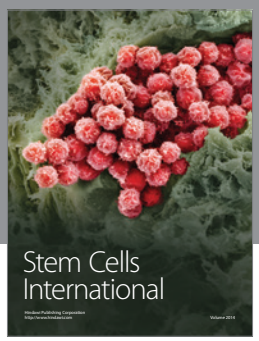

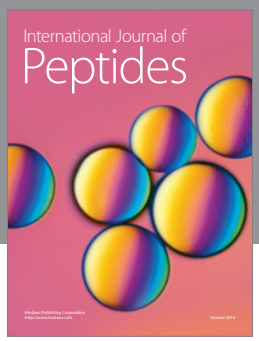

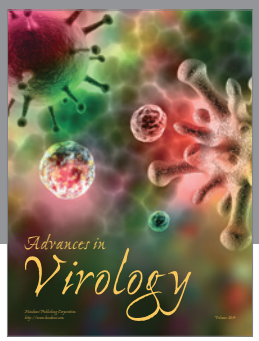

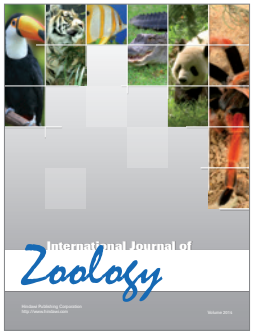

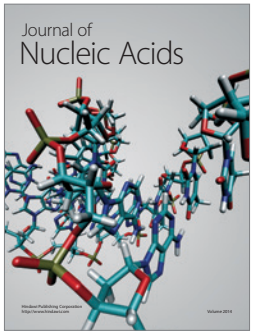

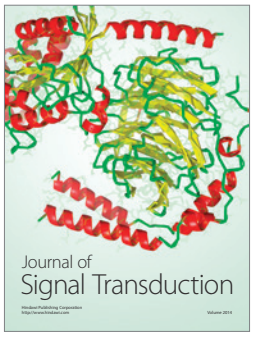

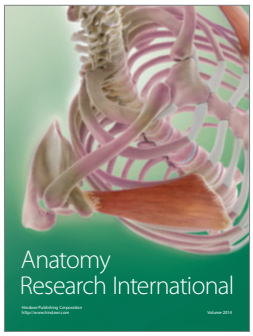

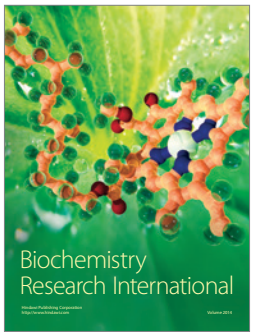

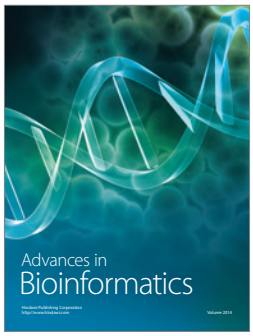

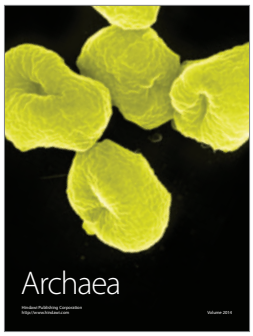

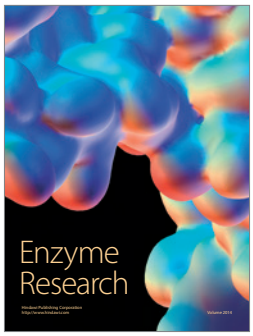

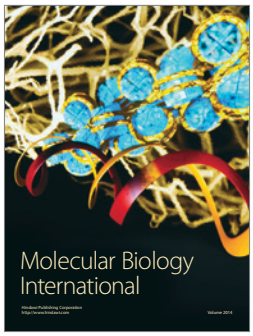

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