Journal of Agriculture and Rural Development in the Tropics and Subtropics
Volume 106, No. 1, 2005, pages 29–39

Evaluation of Nematicidal Action of Some Botanicals on *Meloidogyne incognita* In Vivo and In Vitro

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Abstract
Eggmasses or larvae of *Meloidogyne incognita* were exposed to varying concentrations of neem leaf (fresh and dry), *Borelia* sp., groundnut leaf and garlic bulb. Neem leaf and garlic bulb extracts inhibited hatching of eggmasses and were lethal to larva. A comparative study of neem and garlic bulb extracts prepared at 20% concentration and applied weekly at 25 ml per pot were carried out in the screenhouse. Each pot filled with 2 kg of pasturised soil was inoculated with 2 000 larvae of *M. incognita* by introducing 500 g of infested soil from tomato culture raised in the screenhouse. These extracts significantly reduced root-knot infection indices on tomato when compared to the control. However, garlic extract demonstrated greater potential than neem leaf extract in the control of root-knot infection of tomato in vivo.

Keywords: *Meloidogyne incognita*, neem leaf, garlicbulb, *Borelia* sp., extracts, botanicals

1 Introduction

The use of botanical extracts for controlling *Meloidogyne* is becoming appealing because of the growing problem of environmental pollution arising from the use of persistent pesticides. There has been a de-registration of some hazardous nematicides. Increasing pressure is on farmers to use non-chemical pest control methods that do not pollute the environment. This emphasis the need for new methods of control such as the use of plant extracts. Efficacy of various plant extracts in nematode control has been studied (Akhtar, 1999; Kali and Gupta, 1980; Mukherjee and Sukul, 1978; Netscher and Sikora, 1990; Rossner and Zebitz, 1986). Nematicidal effect of garlic has been reported, but was phytotoxic. Water extracts of some Indian plants and neem leaf were nematicidal on root-knot nematodes and *Pratylenchus* sp. respectively (Mukherjee and Sukul, 1978; Egunjobi and Afolami, 1976). Studies on the identification and use of local plant materials for the control of nematodes, or integrated with other methods of control, are current areas of research in plant nematology . The objective of this study was to (i) evaluate the effect of some botanicals on *M. incognita* (ii) compare the potentials of neem leaf and garlic extracts for the control of root-knot (*Meloiddogyne incognita*) in tomato.

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2 Materials and Methods

2.1 Effect of Extracts of Fresh and Dry Neem Leaves

Fresh neem leaf and dry neem leaf extracts were obtained as follows: 0, 10, 20 and 30 grams of dry neem leaf or fresh neem leaf ground in a mortar were each added to 100 ml of water. The mixture was allowed to stand for four hours and filtered through a fine mesh sieve to remove the leaf debris. The filtrate was further passed through a Whatman No. 40 filter paper overlaid with a 2-ply silktex® tissue paper in a funnel to remove the chlorophyll with a view to having a clear extract of the filtrate. The filtrates were labeled as 0, 10, 20 and 30% concentration of either fresh or dry neem leaf extract.

Twenty *M. incognita* eggmasses were hand-picked with a pair of forceps and introduced into a watchglass to which 1.0ml of 0, 10, 20 or 30% fresh or dry neem leaf extract was applied. Each watchglass with 20 eggmasses received only one concentration level of either fresh neem leaf extract or dry neem leaf extract. Each treatment was replicated three times and the replicates arranged in a completely randomized design on a laboratory bench at a room temperature of between 25 and 27 °C.

The treatments were observed for egg hatchability over a period of 24 hours. After 24 hours, all unhatched eggmasses from each treatment were picked and transferred into a watch glass containing 1.0 ml of tap water. The eggmasses were pierced open and observed for live larvae of *M. incognita*.

Another set of watchglasses containing 20 larvae per watchglass were treated with 1.0 ml of either 0, 10, 20 or 30% neem leaf extract. Each watchglass with 20 larvae received only one concentration level of either fresh neem leaf or dry neem leaf extract at a time. Each treatment was replicated three times and arranged in a completely randomized design on a laboratory bench and observed for larval mortality at 10 minutes interval for 4 hours starting from 10 am.

2.2 Effect of Leaf Extracts of Borelia sp., Groundnut and Garlic

Twenty grams each of fresh leaves of *Borelia* sp. *Borelia* flowers, groundnut leaves and garlic bulb were pounded in a mortar and transferred into a beaker to which 100 ml of water was added. The mixtures were allowed to stand for four hours after which they were filtered through a fine mesh sieve to remove debris. With the exception of garlic, the filtrate was further passed through a Whatman No. 40 filter paper overlaid with a 2-ply silktex® in a funnel to remove chlorophyll.

Twenty *M. incognita* eggamsses were hand-picked with a pair of forceps and introduced into a watchglass to which was added 1.0ml each of the extracts of the fresh leaves of either *Borelia* sp., *Borelia* flower, groundnut leaves or garlic bulb. A control treatment with tap water was included. Each treatment was replicated three times and arranged in a completely randomized design on a laboratory bench at room temperature of between 25 and 27 °C. The treatments were observed for egg hatchability over a period of 24 hours.

2.3 Comparison of Neem and Garlic Bulb on M. incognita In Vivo

Seedlings of wilt susceptible tomato cultivar: Roma VF, were raised on heat pasteurized soil. Three weeks old seedlings were transplanted into plastic (15 cm diameter) pots filled with 2.0 kg of pasteurized soil. Each treatment was inoculated with 2000 larvae of *M. incognita* introduced by adding to each pot 500 cm^3 of root-knot infested soil from tomato culture raised in the screenhouse. There were three tomato seedlings per pot. The fresh neem leaf and garlic bulb extracts were obtained by grinding 20 grams each of fresh neem leaves and garlic bulbs in a mortar to which were added 100 ml of water. The suspensions were allowed to stand for four hours and filtered through a fine mesh sieve to remove the leaf debris. One week after transplanting, 25 ml of the extracts were poured into the soil in the pot around the base of the tomato stem weekly for eight weeks. The experiment was terminated at 10 weeks after transplanting. All treatments were replicated three times.

The following parameters were determined: root weight and shoot weights were determined by carefully removing the plants from the pots and separating into roots and shoots. The fresh roots and shoots were weighed separately. The root-knot galling index was assessed on a scale of 1-10 as described by Bridge and Page (1980). The rating chart used by Bridge and Page (1980) for root galling is as follows:

0 ≙ no knots on roots
1 ≙ few small knots difficult to find
2 ≙ small knots only but clearly visible; main roots clean
3 ≙ some larger knots visible, but main roots clean
4 ≙ larger knots predominate but main roots clean
5 ≙ 50% of roots knotted; knotting on parts of main root system
6 ≙ knotting on some of main roots
7 ≙ majority of main roots knotted
8 ≙ all main roots knotted; few clean roots visible
9 ≙ all roots severely knotted; plant usually dying
10 ≙ all roots severely knotted; no root

The number of eggmasses per gram of root, number of eggs per eggmass and number of adult female per gram of root were assessed by direct counting technique (Daykin and Hussey, 1985). Roots were cut into pieces and thoroughly mixed. Using a Mettler balance, one gram of root was weighed out in three replications. The pieces of root were spread evenly in a grid petri-dish. Adult females and egg masses were counted in each square in the grid. From the one gram of root, three egg masses were randomly picked and transferred into a counting chamber. The eggmass was pierced open with a pair of sharp forceps and the numbers of eggs and larvae were determined. The mean of three replicates was recorded. In the absence of adult females and eggmasses in a root number of larvae per 10 g of root were determined. Roots were teased and allowed to stand over the Baerman's tray for 24 hours. The filterate was decanted into a beaker and nematode population counted over the microscope. Soil analysis for larval population was determined by the modified Cobb's decanting and sieving technique (Barker, 1985). The mean differences between treatment in terms of root-knot infection indices

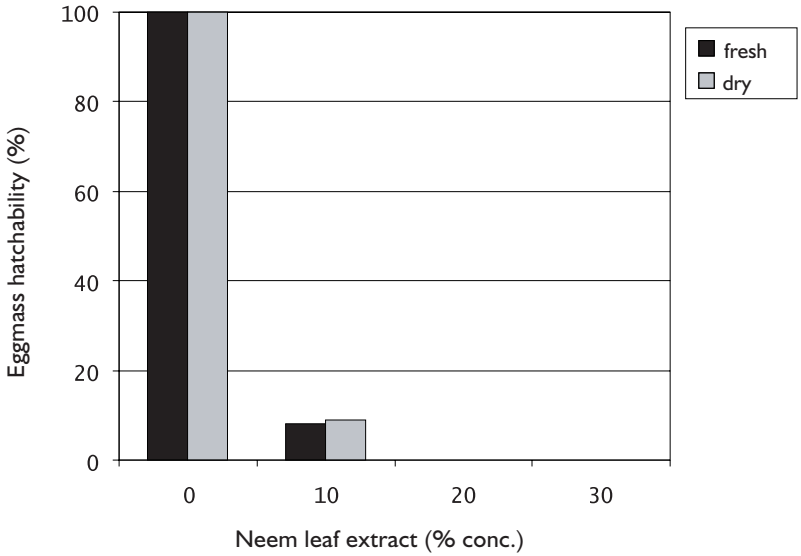
were determined by a non-parametric test of significance, employing the Kruskal-Wallis One Way Analysis of Variance as used by HARRIS and FERRIS (1991), whereas the mean differences of shoot and root weights between treatments were determined by the conventional ANOVA.

3 Results

3.1 Effects of Extracts of the Botanicals on Meloidogyne incognita In Vitro

Every eggmass of *M. incognita* in the control treatment hatched. Hatching commenced between 1-3 hours after exposure to water and was virtually complete after 24 hours. Eggmasses exposed to 20% and 30% concentration of neem leaf extract did not hatch. At 10% concentration of the neem leaf extract, an eggmass ruptured but the released larvae were all deed or moribund (Fig. 1). All unhatched eggmasses teased up contained dead or moribund larvae.

Figure 1: Effect of fresh and dry neem leaf extract on eggmass hatchability at 24 hours after exposure.



The larvae exposed to 20% and 30% concentrations of the fresh neem leaf extracts showed 50% and 90% mortality within 15 minutes of exposure respectively. By 30 minutes, mortality had reached almost 100% in contrast to about 10% mortality recorded in the control after 24 hours (Fig. 2). The effect of fresh neem extract on larval mortality was apparent immediately on exposure.

The dry neem leaf extract caused initial death of larvae only after 30 minutes of exposure to the highest concentration of 30% (Fig. 3). At 10% concentration of the extract, mortality was as low as one larva after 3 hours of exposure. However, after 24 hours of

Figure 2: Effect of fresh and dry neem leaf extract on eggmass hatchability at 24 hours after exposure.

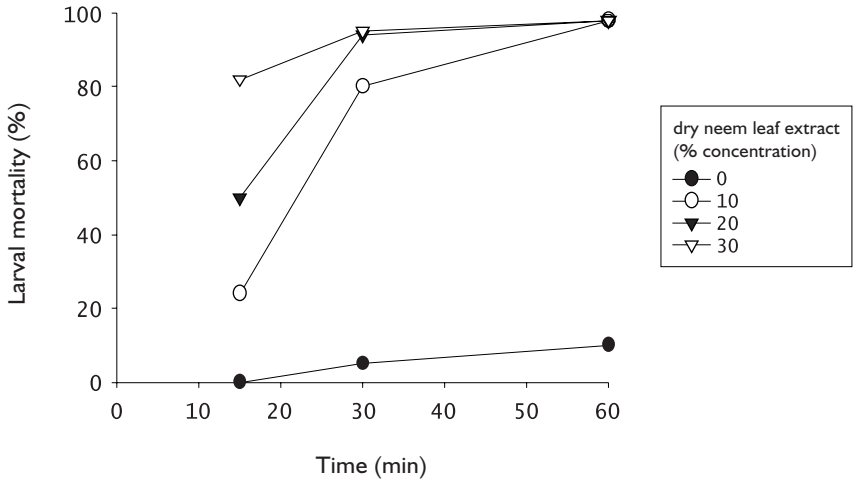
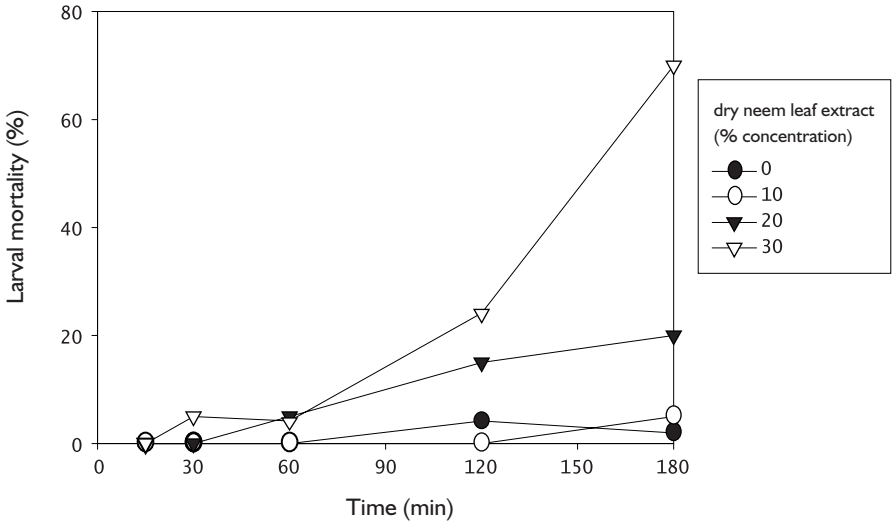


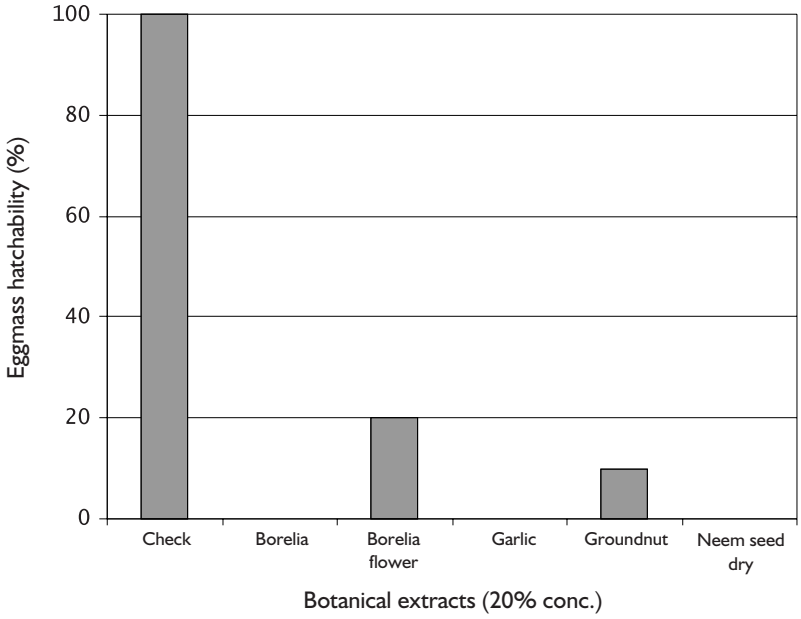
Figure 3: Effect of fresh and dry neem leaf extract on eggmass hatchability at 24 hours after exposure.



exposure all treatment levels of dry leaf extract caused 100% mortality of larvae except in the control where 85% of larvae remained alive. The cumulative effect of the extracts of dry and fresh neem leaves on mortality of larvae and hatchability of eggmasses was similar after 24 hours of exposure regardless of the concentrations of the extracts.

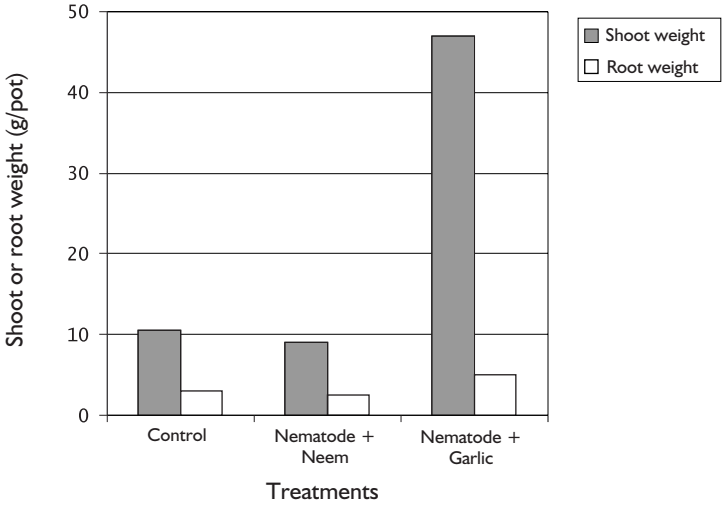
In *Borelia* sp. Flower and groundnut leaf extracts initial hatching of eggmasses occurred after 3 hours of exposure to the extracts in contrast to water where hatching occurred within an hour of exposure (Fig.4). At the end of 24 hours, 100% hatching of of eggmasses occurred in water in contrast to eggmasses exposed to Borelia flower and groundnut leaf extracts where less than 25% of the eggmasses hatched. There was no hatching when the eggmasses were exposed to Borelia leaf extracts (Fig. 4). The larvae in unhatched eggmasses exposed to Borelia flower and groundnut leaf extracts remained motile, while those exposed to Borelia leaf extract were dead or moribund.
Garlic extracts completely inhibited the hatching of eggmasses. All larvae from eggmasses teased open were dead or moribund (Fig. 4).

Figure 4: Effect of fresh and dry neem leaf extract on eggmass hatchability at 24 hours after exposure.



Root and shoot weight differed significantly among treatments, with garlic giving the highest root and shoot biomass (Fig. 5). It was observed that first flowering occurred at 5 WAT, and by the sixth week there was 100% flowering in pots treated with neem leaf and garlic extracts. Root galling index decreased significantly in pots treated with garlic extract. Neither eggmass nor female was obtained from pots treated with garlic

Figure 5: Effect of fresh and dry neem leaf extract on eggmass hatchability at 24 hours after exposure.



extract. There was no significant differences in root galling index between the control and those treated with neem leaf extract. However, eggmass and females per gram of root decreased significantly in neem leaf treated pots compared to the control (Table 1). Similarly, final larval population was significantly lower in garlic and neem leaf treated pots than the control pots. There were, however, larval penetrations of roots but only the second stage larvae were extracted (10 larvae per 10 g of root).

Table 1: fect of neem leaf and garlic extracts on *M. incognita* infection indices on tomato (Roma VF) under screenhouse conditions.

Treatment	*Root-knot galling index*	*Eggmass per gram root*	*Female per gram root*	*Larval population/ per 500* cm^3
Control	7.0^a	43.0^a	45.0^a	1048^a
Nematode + Neem	7.0^a	18.0^b	18.0^b	180^b
Nematode + Garlic	2.0^b	0.0^c	0.0^c	140^b
Signficance level	0.05	0.02	0.02	0.03

Means followed by the same letter in a column are not significantly different ($P = 0.05$) as determined by Kruskal Wallis One Way Analysis of Variance.

4 Discussion

In the present study, there was a complete inhibition of hatching of larvae when fresh eggmasses of *M. incognita* were exposed to neem leaf extracts. A shorter time span was needed for fresh neem leaf extract to obtain similar result as dry neem leaf extract. The initial slow response of larvae to extract of dry neem leaf compared to the fresh leaf is probably due to the slower diffusion of active ingredients into the solution from the dry neem leaves relative to the fresh leaves. Active ingredients in fresh neem leaf extract would more readily diffuse into the water than dry neem leaf extract given the same time span. Hence, after 24 hours the same level of mortality was obtained for both dry and fresh neem leaf extract.

Earlier work in Western Nigeria by EGUNJOBI and AFOLAMI (1976) reported the toxicity of water soluble extracts of neem leaf to *Pratylenchus brachyurus*. Aqueous extracts of leaf, flower, fruit, bark, root and gum of neem were reported to be highly toxic to nematodes with fruit extract showing the most leathal activity followed by leaf extract (PARMAR, 1987). The inability of the eggmass to hatch is as a result of ingress of plant extracts into the eggmass. Larvae in the eggmass were exposed to the toxic effect of the extract resulting first in reduced mobility and finally death or moribund state. Once this state is reached the larva cannot pierce through the wall with its stylet hence hatching ceases. The eggmass which is a part of the perineal region of the femal in root-knot is permeable to the active ingredient in the extracts (HIRSCHMANN, 1985).

Less than 25% of the eggmasses were hatched in the extracts of Borelia flower and groundnut leaf. Furthermore, unhatched eggs contained motile larvae, suggesting that substance in the Borelia flower and groundnut leaves did not inhibit hatching; rather they prolonged the time interval required for hatching. Groundnut plant is known to be resistant to species of *Meloidogyne* occurring in Nigeria (ADESIYUN *et al.*, 1990). Hatching of eggmasses exposed to groundnut leaf extract suggest that resistance of groundnut to root-knot in Nigeria is based on factors other than the presence of toxic substances in the root. Borelia leaf extract exhibited complete inhibition of hatching although extracts from the flower did dot.

The use of botanical extracts for controlling of *Meloidogyne* is appealing because of the growing problem of environmental pollution arising from the use of persistent pesticides like chlorinated hydrocarbons such as chloropicrin. Efficacy of various plant extracts in nematode control has been established. Water extracts of India plants, *Fleurya interrupta, Peritrophe bicalyculata and Andrographis paniculata* were nematicidal and resulted in 100% mortality of root-knot larvae within 40 minutes (MUKHERJEE and SUKUL, 1978). Nematicidal properties of tagetes and wild marigold (TOIDA and MORIYAMA, 1978), *Emblica officinalis* and *Carrissa curandas* (HASEEB *et al.*, 1980) against root-knot larvae have been reported.

Efficacy of plant extracts, however, depends on its concentration and the duration of exposure of the nematode to the extract (MAHMOOD *et al.*, 1979; KALI and GUPTA, 1980). Concentration of active ingredients in neem seed and leaf extracts may differ depending on the environmental condition, the year of collection and geographical area

of the neem trees (Zongo *et al.*, 1993). However, their aqueous extracts appear to contain some nematicidal compounds which tend to inhibit the hatching of eggmass and are directly toxic to *M. incognita* larvae.

In the present study, garlic bulb extract gave significant reduction in root-knot nematode galling index, and root and soil larval populations. The garlic extract showed no phytotoxicity in this study in contrast to some reports of phytotoxicity of garlic extracts to some crop plants (Sukul *et al.*, 1974). Most reports on the use of garlic and neem leaf extracts in nematode control used high application rates and concentrations. (Egunjobi and Afolami, 1976) used 200 ml of 50% of concentration of neem leaf extract per plant to control *Pratylenchus brachyurus* while, Sukul *et al.* (1974) used 250 ml of 50% concentration of garlic extract per plant. These application rates are 8-10 times higher than the application rate of 25 ml of 20% concentration of neem and garlic extracts per plant used in this study. However, such unusually high concentrations may lead to osmotic loss of water from the root tissue resulting in wilting. The relatively low application rate, 25 ml of 20% concentration per plant, used in this study, appears effective against *M. incognita*. Garlic bulb extract was more effective than neem leaf extract as evidenced by the significant reduction in galling index and other reproductive factors such as eggmass/g and number of females/g of root. The results were similar to the findings of Sukul *et al.* (1974) on tomato, that garlic extract was highly effective in reducing root-knot infection. The garlic extract might have been highly lethal to the nematode larvae. The extract probably acted directly on the infective second stage larvae in the soil, thus reducing the number of motile larvae available to penetrate the tomato roots.

Although root-knot galling index was similar in the neem treated and untreated control, the reduction in number of eggmasses, number of females and final larval population of the soil is a strong indication of the ability of neem leaf extract to control root-knot nematode in tomato. Galling and reproductive responses are more reliable indicators of host plant reaction than just root-knot galling index (Fassuliotis, 1985).

The inability of the control plants to flower is probably due to the combined action of the nematode and inadequate availability of nutrients (Netscher and Sikora, 1990). The present study did not record any significant increase in growth among plants treated with aqueous neem leaf extract compared with the control. This is probably due to the low application rate used. Increases in plant growth parameters with aqueous neem extracts have been reported (Egunjobi and Afolami, 1976; Rossner and Zebitz, 1986), and the growth rate depended on the application rate (Kali and Gupta, 1980). Therefore, whereas the low application rate used in this study seemed to give a measure of control of *M. incognita*, plant growth was, however, not affected. Therefore, the productivity of the neem treated plants must be improved by supplementary inorganic fertilizer application or by increasing the application rate. This study shows that garlic has high potentials for nematode control. This, however, needs to be demonstrated under varying field conditions in the savanna.

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