

Full Length Research Paper

Insecticidal properties of materials used by resource-limited farmers to control fleas in free-range chickens in the Eastern Cape Province, South Africa

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Fleas are obligate blood feeders that infest free-range chickens, thereby impeding their productivity. Commercial insecticides used in controlling fleas are expensive and inaccessible, hence making farmers to resort to low cost and easily available alternatives. The study was conducted to assess the insecticidal properties of ethno-veterinary medicine used in controlling fleas in free-range chickens in the Eastern Cape. *In vitro* repellency and contact bio-assay models were used to assess the insecticidal properties of *Tagetes minuta*, *Calpurnia aurea*, *Clutia pulchella*, used engine oil, paraffin, Jeyes fluid (carbolic acid 13%) on fleas. Distilled water was used as the negative control. Positive controls for the repellency and contact bio-assays, were Tabard (35% diethyltoluamide) and Karbadust (carbaryl 5%) respectively. *Tagetes minuta* was the most effective ($P < 0.05$), demonstrating a repellency level of 75 at 100% concentration. *Clutia pulchella*, *C. aurea*, used engine oil and paraffin showed insignificant repellency ($P > 0.05$). The repellency of *T. minuta*, Jeyes fluid and Tabard lasted for 4, 5 and 5 h, respectively. For the contact bio-assay, 100% concentrations of *C. pulchella* and *C. aurea* produced flea mortality of 82.5 and 75%. Paraffin, used engine oil, and Jeyes fluid (19.2%) caused flea mortality, which was not significantly different from Karbadust. The materials assessed showed various degrees of insecticidal properties. This justifies their use by resource-limited farmers in South Africa.

Key words: Fleas, medicinal plants, mortality, repellency.

INTRODUCTION

Fleas are one of the most important external parasites with more than 2000 species and sub-species affecting birds (Boughton et al., 2006). The flea (*Echidnophaga gallinacean*) is a major insect of domestic chickens (*Gallus gallus*) that can cause severe pathology or death if untreated (Boughton et al., 2006). These are blood feeding external parasites responsible for causing ulcerations on soft body parts like the comb and wattles, areas

where the fleas commonly feed (Agboola et al., 2007). The ulcerations are caused by both lacerations from the flea mouthparts as well as resultant infections due to subcutaneously laid eggs. The resulting infection and blood loss, can compromise growth and even kill young chickens (Boughton et al., 2006; Agboola et al., 2007). Additionally, flea infestation has been found to co-occur with fowl pox in chicken, a viral diseases spread by the flea bite (Boughton et al., 2006; Gyimesi et al., 2007). Despite the prevailing negative impact of fleas, which include reduced growth, production and death, most of the resource-limited farmers carry out control measures irregularly. This could probably be due to financial const-

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rains or ignorance (Luseba and Van der Merwe, 2006).

Currently, most flea control efforts by farmers worldwide are based on convenient animal spot-on treatments or oral medications (Power and Sudakin, 2007). The most widely used products are generally applied as spot-on on a monthly schedule (Boughton et al., 2006). Despite the variety of available products and different application methods, fleas remain a perpetual problem for many farmers and have developed resistance to a number of insecticides. As a result, management strategies to reduce the development of resistance are needed (Snyder et al., 2007). This could be addressed by searching for alternative flea control methods that are effective, economical and socially acceptable. Plants produce a diverse array of secondary metabolites with functions, such as defense against diseases and parasites (Pieters and Vlietinck, 2005). As a result natural products, including plant extracts, might be suitable for such alternative approaches (Luseba and Van der Merwe, 2006).

Basically, fleas are controlled by the use of commercial drugs, however use of commercial drugs have limitations such as being harmful to other non-target species and leave persistent residues (Snyder et al., 2007; Power and Sudakin, 2007). This has resulted in resource-limited farmers to use low cost alternative remedies for animal health care, which are cheap, locally available and culturally accepted in their communities. Plant derived products are increasingly being used to combat insects because they are natural and often assumed to be safe to the environment (Pieters and Vlietinck, 2005). Plant derived products may possess complex chemical structures that are not available in synthetic compounds, hence they are potentially useful biologically active compounds (Pieters and Vlietinck, 2005; Isman, 2008). Plants are used as singles or in combinations by resource-limited farmers as reported by Masika and Afolayan (2003) that more than one plant species is combined for the treating livestock in the Eastern Cape. On the other hand, Luseba and Van der Merwe (2006) found that most remedies were used in the form of a single plant. The combinations are intended to attain increased potency due to synergistic interactions between the individual components, such that the combination is superior to individual herbal treatment alone (Adams et al., 2006).

A previous study by Moyo and Masika (2009) revealed that farmers used various materials like Jeyes fluid, and used engine oil to control cattle ticks. Used engine oil is any oil that has served its service properties in a vehicle withdrawn from the meant area of application and considered not fit for its initial purpose (Delistraty and Stone, 2007). However, there is dearth of information on insecticidal properties of materials used in the ethno-veterinary control of fleas. Therefore, the aim of the study was to assess the insecticidal properties of selected remedies used in ethno-veterinary control of fleas in free-range chickens by resource-limited farmers in Eastern Cape, South Africa.

MATERIALS AND METHODS

Plant material collection

The fresh leaves of *Calpurnia aurea*, *Cultia pulchella* and *Tagetes minuta* were collected at Umdeeni village in Amatola Basin (32°40'38"S, 26°59'79"E) in the Amathole District, Eastern Cape Province, South Africa. They were authenticated by Tony Dold at the Albany herbarium, Rhodes University. The leaves of *C. aurea* voucher no. SM01-010/2008, *C. pulchella* voucher no. SM01-011/2008 and *T. minuta* voucher no. SM01-012/2008 specimens were deposited at the Giffen herbarium of the University of Fort Hare.

Preparation of the materials

Aqueous extraction of fresh plant materials

The fresh leaves of *T. minuta*, *C. pulchella* and *C. aurea* were crushed separately using a meat mincer (No 10, Shandong Branch, China). The concentrations of the extracts were determined on a weight per volume basis Yin and Kwok (2005) to obtain 10, 25, 50 and 75% (w/v). The crushed test materials were squeezed to obtain 100% concentration extracts. Plant materials of *C. pulchella* and *C. aurea* were mixed in different proportions of 1:1, 6:4, 7:3, 8:2 and 9:1 to determine the most effective ratio. The mixture was stored at room temperature overnight and later strained using a muslin cloth and filtered using a Whatman No. 1 filter paper, respectively. The filtrates were stored in capped labeled bottles and kept in the refrigerator at 6°C until use.

Preparation of non-plant materials

Jeyes fluid was diluted using distilled water to make the following concentrations: 0.6, 1.2, 4.8, 9.6, 19.2, 38.4, 76.8, and 100%. This was done to determine the effective ratio. Paraffin and used engine oil were used undiluted. The majority of engine oils are derived from petroleum. It mainly consists of hydrocarbons; organic compounds entirely of hydrogen and carbon polycyclic aromatic hydrocarbons are the major constituents of used engine oil (Dominguez et al., 2004). Paraffin also known as kerosene is a hydrocarbon that is highly toxic with a flash point of 43°C (Muller et al., 2007). A commercial insecticide Kabadust (carbaryl 5%) (Agro-serve, Bryanston, South Africa) registered for the control of fleas was used as the positive control and distilled water as negative control.

Fleas

Fleas, *C. felisfelis* at pupae stage were obtained from Clinvet International, Bloemfontein, South Africa. The fleas were held in well ventilated small plastic vial, which was incubated at a temperature of 27 ± 3°C with a relative humidity of 80±10% (Mustapha et al., 2006). After five days, the adult emerged and these 5-day old fleas were used in the experiment.

In vitro repellency bioassay

Test tubes with diameters of 4 cm and lengths of 29 cm were used for this bioassay. The cat fleas, *C. felisfelis* were used because they can also attack chickens and they are the preferred model fleas for repellency testing (Mustapha et al., 2006). Filter papers (Whatman No. 1) with a diameter of lightly less than 4 cm were placed at the bottom of the test tubes. Aliquots of 1 ml of the test materials were applied separately on filter paper as uniformly as possible using a

Table 1. Least square means showing flea repelling activity of *T. minuta* and Jeyes fluid at different concentrations.

Material	Flea repellency (%)								
	20 min	40 min	1 h	2 h	3 h	4 h	5 h	6 h	7 h
<i>T. minuta</i> (100%)	100	100	100	100	100	76	56	36	30
<i>T. minuta</i> (75%)	100	100	100	100	90	66	46	33	26
<i>T. minuta</i> (50%)	100	100	100	100	83	60	36	26	16
Jeyes fluid (100%)	100	100	100	100	100	83	63	50	30
Jeyes fluid (76.8%)	100	100	100	100	86	46	33	20	16
Jeyes fluid (38.4%)	100	100	100	73	43	26	16	13	6
Jeyes fluid (19.2%)	100	100	83	50	30	13	10	0	0
Tabard	100	100	100	100	100	90	70	30	20
Distilled water	0	0	0	0	0	0	0	0	0

micropipette. Distilled water and Tabard (35% diethyltoluamide Acorn (Pty) Ltd, Strubens Valley, South Africa) were used as negative and positive control, respectively. Ten (5 day old fleas) of either sex, anaesthetized using carbon dioxide (CO₂) were placed 8 cm away from test material. The test tubes were closed with fine gauze secured with rubber band and held horizontally for 5 min to allow fleas to gain consciousness. The bottom end of the test tubes was put in a temperature regulated water bath at 41.8°C simulating chicken body temperature so as to attract fleas. Treatments were replicated 3 times. Observations were recorded after 20 and 40 min then hourly for 7 h. Any flea found 4 cm away from the test material upwards was considered having been repelled. Repellency was expressed as number of fleas moving away from the stimuli to the total number of fleas at each occasion;

$$\text{Repellency (\%)} = 100 - \frac{[T \times 100]}{N}$$

Where, T is the number of fleas found in the treated area and N is the total number of fleas used (Usavadee et al., 2007). The average repellency was calculated from the values obtained in three replicates.

Contact bioassay on fleas

The test to evaluate the efficacy of insecticidal activity followed the World Health Organization standard protocols (WHO, 1977) with slight modifications. For the experiment, filter papers were impregnated with test materials at concentrations used in the repellency assay. Distilled water and Karbadust® (carbaryl 5%) were used as negative and positive control, respectively. Ten fleas were anaesthetized with CO₂ and introduced into the test tube 8 cm away from the test material with the aid of a suction tube. Test tubes were closed with fine gauze supported by a rubber band and were held horizontally until the fleas just gained consciousness. Then, the test tubes were held vertically so that the fleas would be in contact with the test material. Test tubes were then incubated for 24 h at a temperature of 27 ± 3°C with a relative humidity of 80 ± 10% (Mustapha et al., 2006). Mortality observations were done after 24 h. The mortality rate was calculated according to Abbot (1925) as follows:

$$\text{Flea mortality} = \frac{(1 - T)}{Co} \times 100$$

Where, T is the number of live fleas after treatment with test material; Co is the number of live fleas after treatment with control. Dead adult fleas were identified when they failed to move after probing with a needle (Maheswaran et al., 2008).

Statistical analysis

In vitro repellency bioassay

The percentage flea repellency was calculated according to Usavadee et al. (2007). Mean repellency for all materials used was calculated. Data were analysed using Proc mixed for repeated measures (SAS 2002) to compute statistical differences of least square means.

Contact bio-assay

Mortality counts were corrected by using the formula of Abbot (1925). Data obtained were tested for normality before analyzed using SAS (2002). The General Linear Models (GLM) procedure was used to analyse the effect of treatment on fleas. Comparison of means was performed using the Tukey's student range test.

RESULTS

In vitro repellency bioassay

Tagetes minuta demonstrated a significant repellency (P<0.05) at a concentration of 75% at a concentration of 100% for 4 h (Table 1). Extracts of *C. aurea*, *C. pulchella* (10, 25, 50, 75 and 100%) and their proportions of 1:1, 6:4, 7:3, 8:2 and 9:1, used engine oil and paraffin did not show (P>0.05) repellency. Jeyes fluid at concentrations of 38.4, 76.8 and 100% lasted for 2, 3 and 5 h, respectively, while the standard Tabard was effective for 5 h after application (Table 1). The repellency efficacy of test materials decreased with time. Differences of least square means with time are reflected on Table 2. Extracts of *T. minuta* at 50, 75, and 100% concentrations showed significant difference at 3 to 4 h range. Jeyes fluid at 76.8% compared well with Tabard which showed the significant difference at 4 to 5 h range.

Table 2. Differences of LSM with treatment and time.

Material	Time frame range (h)							
	0.33 - 0.67	0.67 - 1	1 - 2	2 - 3	3 - 4	4 - 5	5 - 6	6 - 7
<i>T. minuta</i> (100%)	-124E-16	2.31E-14	1.78E-14	-551E-16	23.3**	20**	20**	16.7
<i>T. minuta</i> (75%)	-115-16	0	2.75E-14	10	23.3**	20**	13	6.6
<i>T. minuta</i> (50%)	-191E-16	1.11E-14	1.95E-14	16.7	23.3**	23**	10	10
Jeyes fluid (100%)	-213-16	0	2.31E-14	-249E-16	16.7	20**	13.3	20
Jeyes fluid (76.8%)	-147E-16	-107E-16	3.73E-14	13	40**	13	3.3	13
Jeyes Fluid (38.4%)	-213-16	5.33-15	26.7**	30**	16.7	10	3.3	6.67
Jeyes fluid (19.2%)	-213-16	16.7	33.3**	20**	16.7	3.3	10	-155E-16
Tarbard	3.13E-13	-256E-15	7.11E-4	-128E-15	10	20**	40	10

**Differences of LSMs in each row with the two stars are significantly different at ($P < 0.001$). *Standard error (± 4.4)

Table 3. Mean percentage mortality (\pm S.E) after 24 hour exposure of fleas to the plant material.

Material	Concentration (%) (w/v)				
	10	25	50	75	100
<i>T. minuta</i>	2.5 ^q	7.5 ^q	20 ^{opq}	32.5 ^{mno}	42.5 ^{ijk}
<i>C. aurea</i>	30 ^{nop}	37.5 ^{klm}	50 ^{ghl}	67.5 ^{def}	75 ^{bc}
<i>C. pulchella</i>	15 ^{pq}	30 ^{nop}	62.5 ^{def}	72.5 ^{cd}	82.5 ^{ab}
<i>C. aurea</i> + <i>C. pulchella</i> 1:1	20 ^{opq}	42.5 ^{ijk}	56.2 ^{efg}	60 ^{efg}	72.5 ^{cd}
<i>C. aurea</i> + <i>C. pulchella</i> 8:2	20 ^{opq}	45 ^{hij}	50 ^{ghl}	65 ^{def}	70 ^{cd}
<i>C. aurea</i> + <i>C. pulchella</i> 9:1	36.3 ^{klm}	51.3 ^{ghl}	56.3 ^{efg}	67.5 ^{def}	72.5 ^{cd}
<i>C. pulchella</i> + <i>C. aurea</i> 8:2	30 ^{nop}	45 ^{hij}	55 ^{efg}	65 ^{def}	70 ^{de}
<i>C. pulchella</i> + <i>C. aurea</i> 9:1	35 ^{lmn}	50 ^{ghl}	65 ^{def}	70 ^{de}	72.5 ^{cd}
Karbadust	-	-	-	-	97.5 ^a
Control	0 ^q	0 ^q	0 ^q	0 ^q	0 ^q

Mortality means for each test material with the same superscripts are not significantly different at $P < 0.001$ level. *Standard error (± 3.60).

Contact bioassay

The plant extracts of *C. pulchella*, *C. aurea* and *T. minuta* at concentration of 100% had flea mortality of 83.5, 73.3 and 42.5%, respectively, while the combination of *C. pulchella* and *C. aurea* in proportions of 1:1, 8:2 and 9:1 had mean mortality of more than 70% at higher concentrations (Table 3). Other proportions 6:4 and 7:3 of both plants showed insignificant flea mortality compared to single plant extracts. Paraffin and used engine oil exhibited flea mortality of 92.5 and 95%, respectively, and were not significantly different ($P < 0.05$) to the positive control Karbadust (carbaryl 5%) that exerted a mean mortality of 97.5%. Jeyes fluid at 19.2, 38.4 and 76.8% concentration had a flea mortality of 82.5, 95 and 100%, respectively. Lower concentrations exhibited insignificant flea mortality efficacy. Although farmers use Jeyes fluid to control fleas, however, in this study the concentration level of 1.2% which is used for general disinfection did not exert significant flea mortality. Other proportions 6.4 and 7.3 of both plants showed insignificant flea mortality compared to single plant extracts.

DISCUSSION

Insecticidal activity of ethno-veterinary medicine against several insects has been demonstrated (Isman, 2008) and the deleterious effects of these on insects can be manifested in several ways, which include toxicity, mortality and repellency (Jbilou et al., 2006). In this study, *T. minuta* exhibited both insecticidal and insect-repellent properties. This is in agreement with Cestari et al. (2004) who also documented that *T. minuta* possesses both insecticidal and repellent properties, though the insecticidal activity of *T. minuta* was insignificant in the current study. The insecticidal activity of *T. minuta* has been reported against stored product pests (Sarin, 2004) and human head lice (Cestari et al., 2004). Its efficacy could be attributed to its composition as the main oil constituents recorded α -terpineol and ocimenone (Liza-lopez, 2008). The α -terpineol and ocimenone in *T. minuta* are responsible for the toxic and repellent effects reported in flies (Njoronge and Bushmann, 2006). Generally, oil extracts are more effective than the aqueous (Sadad, 2011) because water extracts which is different from other sol-

vents do have myriads of compounds that act antagonistically in their overall activities (Boussaada et al., 2008). Nevertheless, in this study, *T. minuta* did not record significant flea mortality. This may be attributed to the fact that aqueous leaf extracts, and not the oil were used. However, it is easy for resource-limited farmers to use aqueous extracts than *T. minuta* oil.

In this current study, *T. minuta* showed significant repellency against fleas. This could be attributed to the pungent smell which repelled fleas away from the test material. The decrease of *T. minuta* repellency efficacy with time could be due to the fact that it contains volatile substances. Zorloni (2007) reported hexane extract of *T. minuta* having a repellency efficacy of 82% against ticks for 2 h at a low concentration of 10%, and this may be attributed to the fact that hexane not aqueous extracts were used. According to Fradin and Day (2002), the minimum repellency efficacy of commercial insecticides is 100% for 2 h and the natural repellent products is 100% for the first 30 to 60 min. *Tagetes minuta* at 50, 75 and 100% and Jeyes fluid at 76.8 and 100% had a repellency efficacy of 100% for more than 1 h, and certainly these conform to the requirement by Fradin and Day (2002). This duration compares well with the required 2 h as there is no significant difference between the two durations and as such these could be used by resource limited farmers as alternative flea repellents. Reapplication of the test material at 2 h interval is needed for the test materials to be effective for several hours. This requires more material, laborious, labour intensive, and consumes more time.

Jeyes fluid is a commercial product used as a household disinfectant. It contains mainly tar acids-13%*m/m* (carbolic acid) and sodium hydroxide (1%) (Henriette, 2012). It showed some insecticidal activities both through contact and repellency in the study. The effectiveness of Jeyes fluid in killing fleas is attributed to poisonous carbolic acid (Henriette, 2012) and its efficacy compared well with the positive control Karbadust (carbaryl 5%). Jeyes fluid has been used for many years as an alternative method to control ticks (Hlatshwayo and Mbat, 2005). It is accessible to resource-limited farmers and they perceive it cheaper and easy to use compared to the commercial insecticides (Hlatshwayo and Mbat, 2005); this could be the reason why rural farmers use it to control fleas. So far, no reports about its repellency properties against fleas have been documented. The Jeyes fluid is very toxic to aquatic organisms, as it alters the water pH. This may cause long term effects in the aquatic environment if water bodies are contaminated (Henriette, 2012). Its use should be avoided or used with caution to avoid contamination of water bodies.

In this study, used engine oil exhibited significant flea mortality. It contains toxic metals such as aluminium, chromium, lead, and manganese (Delistraty and Stone, 2007) and its efficacy could be due to these toxic substances contained. The toxic and carcinogenic substances

contained in used engine oil can cause negative effects to the ecosystem (Delistraty and Stone, 2007). Despite the negative effects previously mentioned, Hlatshwayo and Mbat (2005) reported that used engine oil is being used as an alternative tick control by resource-limited farmers because of its accessibility. However, despite its wide use as an alternative remedy in the control of external parasites it could be used with caution. In addition, paraffin also showed some significant mortality against fleas, however, little or no literature has documented its efficacy against external parasites. Previous studies reported its use by farmers to control fleas (Muchadeyi et al., 2004) and ticks (Hlatshwayo and Mbat, 2005). Paraffin is composed of sulphur, olefins, aromatics and aliphatics (Dlamini and Gqaleni, 2006). Aromatics are associated with high toxicity to skin tissues (Dlamini and Gqaleni, 2006) and they induce necrosis on the skin. These include toluene, ethylbenzene, p-xylene and phenylmethane, which are commonly found as industrial solvents in the manufacturing of paints, chemical, pharmaceuticals and rubber (Broberg et al., 2008). Although it is widely used by resource-limited farmers in most developing countries, it has to be used with caution.

In this study, *C. pulchella* had the highest flea mortality percentage among plants. However, information is lacking on its previous use against fleas and its insecticidal properties. It has previously been reported to be used in drenches for griping pains in calves (Hutchings et al., 1996) and its decoction to treat gall sickness (Masika and Afolayan, 2003). *Calpurnia aurea* also exhibited significant flea mortality at higher concentrations. Some findings by Adedapo et al. (2008) revealed that *C. aurea* (Ait) Benth is used to kill lice. In a study by Zorloni (2007), *C. aurea* exhibited high tick mortality. This could be due to the fact that acetone and not aqueous extracts were used. Also, active compounds from *C. aurea* such as quinolizidine alkaloid calpurnine (12 β , 13 α dihydroxylupanine) and its 13 α -(2-pyrrolylcarboxylic acid ester) have been reported by Zorloni (2007). These quinolizidine alkaloids are toxic to insects and animals (Adedapo et al., 2008). Their toxicity could have attributed to the mortality of fleas. Insecticidal properties of *C. aurea* have also been recorded by Blum and Bekele (2002). In another study, Regassa (2000) reported the use of juice from crushed leaves of *C. aurea* in the control of ticks. Furthermore, its insecticidal activity against lice in calves has been reported by Heine and Brenzinger (1988) in Kenya. The wide use of *C. aurea* could justify its use by resource-limited farmers of Amatola basin in controlling flea.

This study revealed that combination extracts of *C. pulchella* and *C. aurea* did not exhibit significant repellency against fleas. The result could be attributed to the observation that both plants did not possess repellent properties or possible that there may have antagonistic effects. In contact bioassay, the proportions of 1.1, 8.2 and 9.2 had significant flea mortality at the highest concentration, but it was less than when single extracts were

used. This could be attributed to the fact that the compounds in each extract in plant combinations may neutralize each other (Gilani and Rahman, 2005). Also, in some instances the combination may result in antagonistic effects (Adams et al., 2006); this could explain the effect of two extracts being less than that of a single extract. According to Adams et al. (2006), combining two plants extracts increase potency due to synergistic effect. However, it is hard to predict synergistic and antagonistic effect because in this study, the concentration of active ingredient of plant extract was not estimated. Further investigations are, therefore, recommended in this regard. It was also noted that there were no significant differences in mortality when using different proportions of *C. pulchella* and *C. aurea* and this could necessitate finding the compounds that could contribute to this effect.

Amongst the materials used by resource-limited farmers and reported herein, most of them possess insecticidal and repellent properties against fleas. Despite the fact that some materials showed effectiveness against fleas, used engine oil and Jeyes fluid have potential toxic effects in animals and are also environmental contaminants. Hence, it is recommended that those using it should exercise extreme care and caution

Conclusion

The present investigation established that *T. minuta* and Jeyes fluid showed some repellency efficacy against fleas, while *C. pulchella*, *C. aurea*, paraffin and used engine oil did not exhibit any efficacy at all. At the same time, *C. pulchella*, *C. aurea*, paraffin, used engine oil and Jeyes fluid showed some flea mortality efficacy. Plant combinations of *C. pulchella* and *C. aurea* in proportions of 6:4 and 7:3 did not exhibit significant flea mortality at low concentrations. This work demonstrated some of the tested materials to be very effective in the repelling and killing of fleas. However, further investigations are recommended to determine flea insecticidal compounds of *C. pulchella* and *C. aurea*. Despite the wide use of used engine oil, it is an environmental contaminant; therefore farmers need to exercise caution when using it.

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