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Insecticidal and Insect Reproductive Inhibition Potential of Citrus Peel Powder on *Tribolium castaneum* (Herbst)

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ABSTRACT

Insecticidal efficacy of citrus (Citrus spp) peel powder (CPP) against Tribolium castaneum (Herbst) was carried out in the Department of Zoology and Environmental Biology, University of Calabar, Nigeria. Treatments constituted CPP (orange, tangerine, lemon, grape and lime) admixed separately with maize grain (popcorn) at different dosages of 1, 2, 3, 4, and 5 g per 50 g equivalent to 2, 4, 6, 8, and 10 % w/w laid out in completely randomized design with four replications. Parameters measured included mortality, larval development, adult emergence and weight loss. Sweet orange and Tangerine at 8 and 10 % w/w were efficacious compared to other treatments as they significantly caused higher mortality of T. castaneum, reduced oviposition, larval development, and inhibited adult emergence as well significantly reduced weight loss. Increase in concentration resulted to increase in efficacy. Therefore, Tangerine and sweet orange peel powders can serve as a protectant against T. castaneum at a higher concentration.

Key words: Citrus peel, *Tribolium castaneum*, mortality, oviposition, popcorn

INTRODUCTION

The red flour beetle *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae) is a primary pest of flour and other milled products of cereals and a secondary pest of stored wheat (Irshad and Talpur 1993, Suresh and White 2001) causing severe damage to these food grains both quantitatively and qualitatively (Smith *et al*., 1971). The species can be a major pest in anthropogenic structures used for the processing and storage of grain based products such as flour, mills, warehouse, retail stores etc. This species has a long association with human stored food and has been found in association with a wide range of commodities including grain flour, peas, beans, nuts, dried fruits (Chanbang *et al*., 2008). Besides these, members of the genus *Tribolium* are reported to secrete certain toxic quinones of carcinogenic nature in stored commodities thus posing serious risks to human health (El-Mofty *et al*., 1989). *T. castaneum* has been found to invariably infest groundnut kernels and pods causing substantial loss in storage because of its high reproductive potential (Prakash and Rao, 1997). The beetles can breed throughout the year in warm areas. Losses caused by this pest could be prevented either by use of pesticides or by non-chemical methods. Chemical methods involve the use of synthetic insecticides in most parts of the world (Okweche *et al*., 2013). The use of synthetic pesticides to control these pests is highly discouraged because of their adverse effects on human health and the environment. Plants are known to be good sources of grain protectants (Prakash and Rao, 2006). However, at present, there is an emphasis on the application of reduced risk insecticides such as methoprene (Arthur and Fontenot, 2012).

The effectiveness of methoprene against many insect species, including *Rhizopertha dominica* (Fabricius), *Cryptolestes ferrugineus* (Stephens), *Oryzaephilus surinamensis* (L.), and *T. castaneum* have been reported in literature (Nayak *et al*., 1998, Chanbang *et al*., 2008, Arthur and Fontenot, 2012). Similarly, the effectiveness of botanical insecticides has been demonstrated in many studies (Aslan *et al.,* 2005; Cetin and Yanikoglu, 2006; Negahban *et al.,* 2007; Ayvaz *et al.,* 2009; Ayvaz *et al.,* 2010; War *et al.,* 2014). The plant powders are highly repellent and they can play a role in storage protection of stored grains (Ayvaz *et al.,* 2010; War, *et al.,* 2014). Many of the plants used to preserve crops have been found to be safe for human consumption. Several number of plant products in various forms have been screened against major pests of stored grain and their effects are on the pests are on secondary metabolism, act as ovipositional deterrents, feeding deterrent and growth retardants (Ukeh *et al*., 2012).

Most of the plant products are non-pollutants, less toxic and are highly biodegradable in nature (Jilani, 1984; Omotoso, 2014). Hence in the present investigation, different citrus peels were used as bioinsecticides in powder form for the management of *T. castaneum.* The research is therefore aimed at determining the efficacy of citrus peels for the management of *T. castaneum*

MATERIALS AND METHODS

Collection and preparation of citrus powders

Different fresh citrus fruits of sweet orange (Citrus sinensis), tangerine (C. reticulata), lemon (C. limon). Grape (C. paradisi) and lime (C. aurantifolia) were bought from Marian Market, in Calabar, Cross River State. The identity of the fruit was confirmed from the herbarium room in the Department of Botany, University of Calabar, Calabar. The citrus fruits were peeled using a sharp clean knife and the peels were shade dried naturally outside the laboratory until they were crisp dry. Powder was prepared by grinding each of the citrus peels separately in a sterilized electric grinder, and then passed through a mesh size of 0.5 mm to obtain fine and uniform sample. The resulting plant powders were stored separately in white transparent container with label and kept at ambient laboratory temperature prior to use.

Collection and preparation of maize grain (popcorn)

Maize grains (field corn) were obtained from Watt Market in Calabar, Cross River State. All maize seeds were inspected and separated to avoid mixing the corn that had already been infested with *T. castaneun* with the uninfested ones. The resultant uninfested corns were then preserved in a sack bag pending when needed.

Insect culture

Culture of the test insect was raised from naturally infested maize grains bought from Watt market, Calabar, Cross River State. The weevils were reared on uninfested maize under fluctuating ambient temperature (25-30^{o}c) in the laboratory inside a white transparent container and covered with mesh net to permit ventilation. The mesh net was held in place with rubber bands.

Toxicity assays

The pulverized plant powder of citrus peels was dry-mixed separately inside rearing containers with maize at 0, 1, 2, 3, 4, 5 and 6 g of citrus powder respectively per 50g of the maize and was shaken for 2-5minutes to ensure homogenous mixture. Ten pairs of unsexed 24 hours old *T. castaneum* adults were obtained from the cultures and introduced into each of the containers, including the control (untreated). The containers were then covered with mesh net held in place with rubber bands. The treatments were replicated three time. The experiment was arranged in a completely randomized design.

Data collection

Data on the following parameters were recorded: adult mortality at 7 days' intervals, larval development, effects of the powder on adult emergence and insect productivity after 30 days, number of feeding holes and grain weight loss.
Adult mortality was determined at 7 days and 14 days' interval. Dead weevils were carefully removed from the container using a pair of forceps. Percentage adult mortality was determined by using the formula (equation 1):

$$Mortality\ (\%) = \frac{\text{No.of dead insect}}{\text{Total no.of insect introduced}} \text{ x } 100$$

Adult emergence

Adult weevils which emerged at the end of the observation period of 30days were identified, counted and recorded.

Grain weight loss

The grains were weighed before and after attack by the weevil using electronic weighing balance. Weight loss of the grains (popcorn) was determined after the 30 days' observation period using the formula (equation 2):

$$\%\ weight\ loss = \frac{\text{Initial weight of maize} - \text{Final weight of grain}}{Iniial\ weight\ of\ maize} \text{ x } 100$$

Data analysis

Data obtained were transformed using square root transformation and then subjected to one–way analysis of variance (ANOVA) following Completely Randomized Design. Significant means were separated using Fishers Least Significant Difference at 5 % level of significance.

RESULTS

The results showed significant ($p < 0.05$) effects of treatments and levels of applications on the various parameters assessed (mortality, number of feeding holes, adult emergence, insect productivity and grain weight loss). The effects of citrus peel powder on the mortality of *T. castaneum* showed that sweet orange and Tangerine peel powder significantly increased mortality rates compared with other treatments (Fig. 1). The general trend was that mortality increased with increase in the concentration of the citrus powder. 10 g concentration of sweet orange,

tangerine and lemon were significantly more effective compared with 4, 6 and 8 g concentrations. Also, 4, 6 and 8 % concentration of sweet orange and tangerine peel powder were as effective as grape and lime peel powder at 10 and 12 % concentration (Fig. 1). Sweet orange and tangerine peel powder treated Petri dishes had significantly lower number of feeding holes, which was followed by lemon peel powder while grape and lime peel powder had significantly higher number of feeding holes compared with other treatments. Untreated (0 %) seeds had significantly higher numbers of feeding holes. Irrespective of the citrus species, application of 4, 6, 8 and 10 w/w of the citrus peels powder had significantly reduced number of feeding holes compared with 0 and 2 g (Fig. 2).

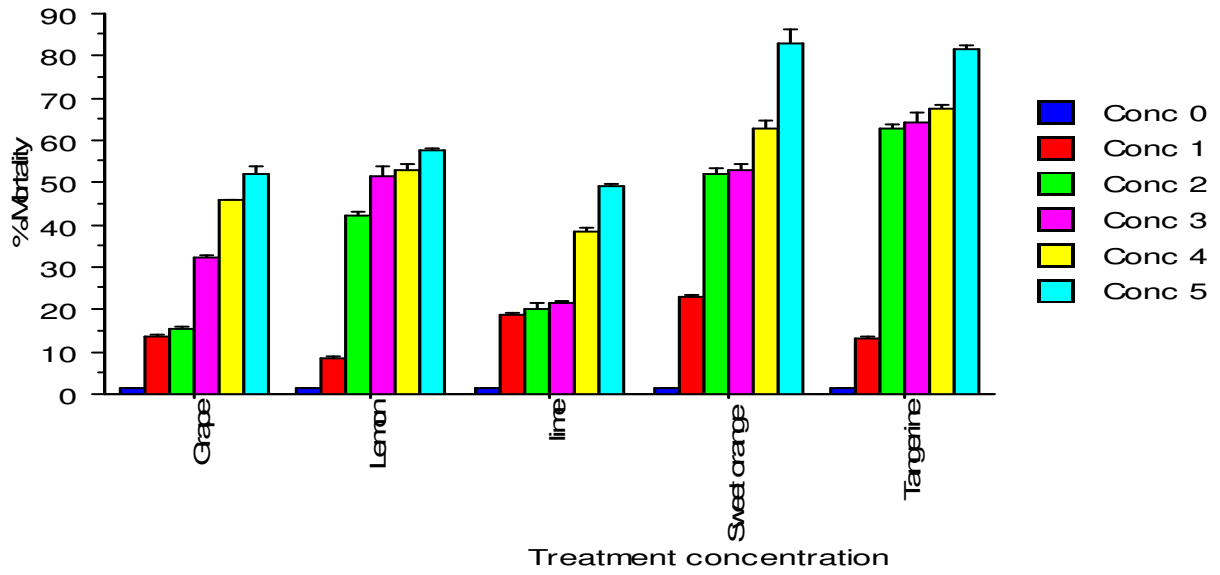

Figure 1: Figure 1: Effects of different concentrations of citrus peel powder on adult mortality of *T. casterneum*
Key: Conc 0, 1, 2, 3, 4, 5 = 0, 2, 4, 6, 8 and 10 per cent w/w, respectively

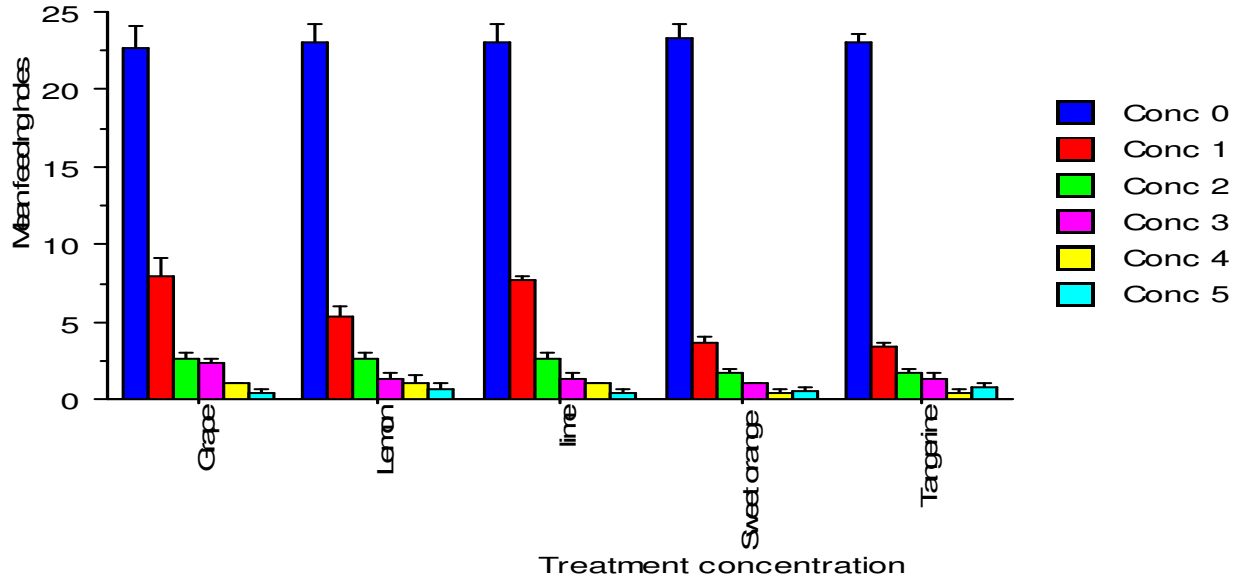

Figure 2: Effects of different concentrations of citrus peel powder on on mean number of feeding holes created by *T. casterneum*
Key: Conc 0, 1, 2, 3, 4, 5 = 0, 2, 4, 6, 8 and 10 per cent w/w, respectively

The application of the different types of citrus powder significantly reduced the number of adult emergence. Application of 0 g of citrus peels powder had significantly higher adult emergence compared with 2, 4, 6, 8 and 10 % w/w application (Fig. 3). However, significant ($p<0.05$) differences existed between the different levels of application with the control (0 % application) recording significantly higher number of emerged adults compared with other levels of applications, although no significant differences existed between 8 and 10 % which were highly effective compared with other levels (Fig. 3). Insect productivity was affected by the application of the different powders. There was no significant ($p>0.05$) difference between the potency of lemon, sweet orange and tangerine peel powders which were found to significantly reduce insect productivity compared with lime and grape peel powders. Application of 10 % citrus powder was significantly ($p<0.05$) effective in all the assessed parameters compared with other levels of application; this was followed closely by 10 % and 8 % of citrus powders (Fig. 4).

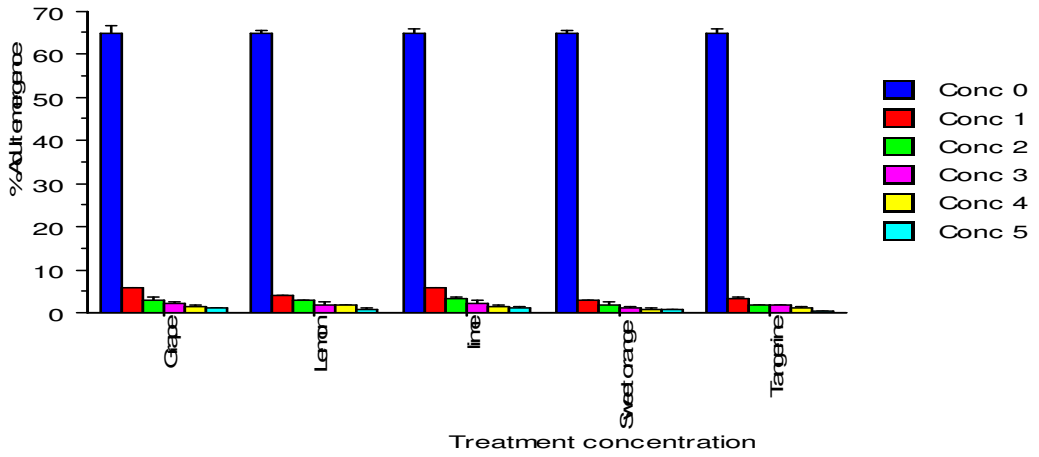

Figure 3: Effects of different concentrations of citrus peel powder on number of adult emerged. Key: Conc 0, 1, 2, 3, 4, 5 = 0, 2, 4, 6, 8 and 10 per cent w/w, respectively

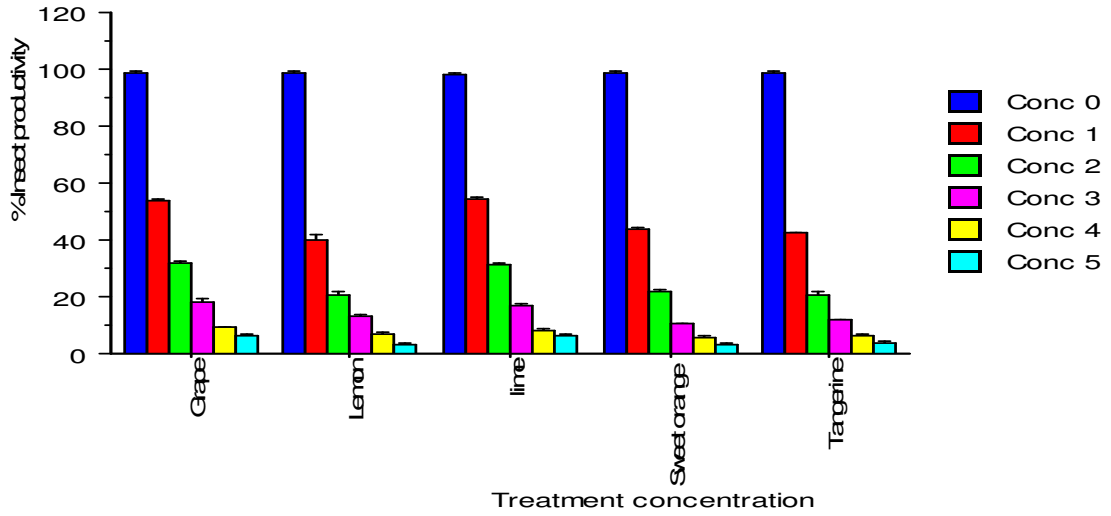

Figure 4: Effects of different concentrations of citrus peel powder on mean insect productivity. Key: Conc 0, 1, 2, 3, 4, 5 = 0, 2, 4, 6, 8 and 10 per cent w/w, respectively

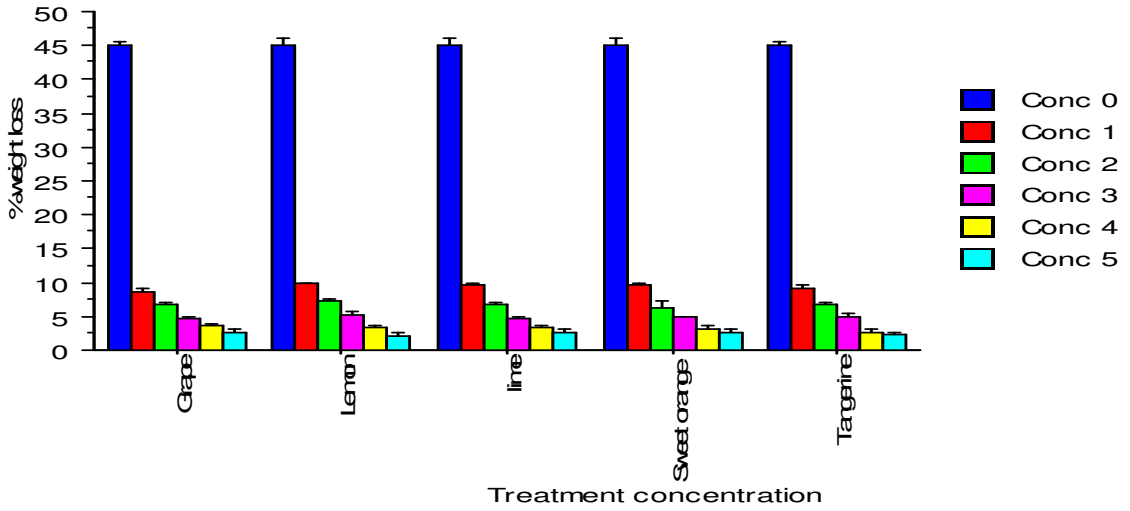

Figure 5: Effects of different concentrations of citrus peel powder on mean percentage weight loss. Key: Conc 0, 1, 2, 3, 4, 5 = 0, 2, 4, 6, 8 and 10 per cent w/w, respectively

Weight loss was affected by the application of the citrus peel powder. The application of citrus powder to the maize seeds had significant ($p<0.05$) effects on the parameters assessed. Untreated seeds had significant ($p<0.05$) higher reduction in weight compared with other citrus peel powder concentration levels. The application of 10 citrus species powder was significantly ($p<0.05$) more efficacious than 2, 4, 6 and 8 % concentrations as well as the control (Fig 5).

DISCUSSION

The high insecticidal potency of citrus peel powder in the control of the voracious attack of *T. castaneum* on stored grains as indicated in this study is not surprising. A review of literature on the effect botanicals employed in the control of insect pests has revealed that citrus peels are highly toxic to stored grains pests. Taylor (1975) and Omotoso (2014) reported that citrus oils from lemon, grape fruit, lime, kumguat and tangerine applied topically were highly toxic to *Callosbrachus maculatus and Sitophilus oryzae,* which are in agreement with results of this study. They also reported that an application of extracted lyophilized oils to the surface of black eyed peas (cowpea) prevented the development of progeny of *C. maculatus*. Naima *et al* (2013) reported that bruchid larvae did not develop in pea flour containing 0.1 – 0.5% of the different citrus oils, which is in conformity with the high oviposition inhibition properties of citrus peels in this study. The mortality of *T. castaneum* caused by citrus peels powder could be attributed to several chemical metabolites such as terpenes (limonene found in citrus) and hydrocarbons which exhibits both toxic and repellent effect on the test organism (Taylor,1975). Consequently, the biological action of natural products with insecticidal activity is a very important alternative, which allows an environment-friendly management of pest insects without affecting human health. Plants produce a wide diversity of compounds involved in their chemical defence. Among these natural products, terpene compounds have been shown to have a significant potential for insect control (Toloza, *et al*., 2010; Lardeux, *et al*., 2010). However, little is known about the molecular properties related to their insecticidal activity.

Result from this research has indicated that orange fruit peel powder was the most potent and highly toxic resulting in high mortality of *T. castaneum*. This result agrees with an earlier report by Dawit and Bekelle (2010) who reported that orange peel oil at high concentration level of 750 mg litre^{-1} applied at 3 ml caused 100 per cent mortality, of *Zabrotes subfasciatus* after four days of application. Sharaby (1988) and Tripathi *et al.* (2003b) showed that the insecticidal activities of *C. sinensis* (orange peel) may be attributed to a chemical metabolite called d-limonene. They added that d-limonene with LD_{50} 74.73, 85.37 and 79.78 showed contact toxicity to *Rhyzopertha dominica, S. onyzae* and *T. castaneum.* The study also agrees with reports of Belmain and Stevenson (2001) on the effective use of *C. sinensis* powder against legume pests. They attributed the effectiveness of their extracts to silica-like components, which are abrasive and have the ability to

adhere to the grain. Furthermore, the use of citrus powder as protectant could have resulted to death of the pest given the tendency of the powder to block the spiracle of the insect, thus impairing respiration, leading to the death of the insect which agree with the reports of Levinson *et al.* (2003) and Owoade (2008). All treatments caused significant reduction in F_1 adult emergence compared to untreated experiment, but the extent to which orange fruit peel powder affected the survival of the subsequent progeny were found to vary among other citrus peels. The present finding is similar to the results of Tripathi *et al.* (2003a) who have reported 94.5 per cent reduction in toviposition rate of *T. castaneum* after application of orange peel oil. Similarly, Sharaby (1988) reported reduced oviposition and egg hatching of potato tuber moth, *Phthorimaea operculella* exposed to 220ml of the orange peel oil. It was also reported by Levinson *et al.,* (2003) that orange peel oil at 1ml suppressed oviposition of Mediterranean fruit fly, *Ceratitis capitata*. This indicates that plants powder (citrus peel powder) have some toxic factors which negatively impacted the insect survival. Significant reduction in weight as a result of damage caused by *T. castaneum* indicates higher protectant potential ability of these materials (i.e. citrus peels) against damage of the pest. Ömer *et al*. (2004) had reported the anti-feedant and toxicity effects of some plant extracts on the larvae and adults of *Onyponomeuta malinellus.* Equally, Masumeh and Zahra (2016) reported significantly higher mortality of *Rhyzopertha dominica* and *T. confusum* when exposed to maize treated with three different formulations of silica nanoparticles. The plant materials were highly effective and significantly reduced damage to maize grains when compared to the control (untreated experiment).

CONCLUSION

Citrus peels have insecticidal properties which account for much higher levels of effectiveness. Furthermore, orange peels powder at 10 % w/w applied to maize grains also reduced the damage to grains by *T. castaneum*, hence, strongly suggesting the presence of interfering agents in orange peel powder. Besides, orange peels are readily available, cheap and does not require robust processing to obtain the extracts, hence can be recommended for use by the poor resource farmers.

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