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Laboratory test of cigarette butt waste and soursop Leaf (*Annona muricata* L.) extracts as biopesticides of fall armyworm (*Spodoptera frugiperda*)

Syadza Firdausiah^{1*}, Firdaus¹, Sulaeha Thamrin², Muhammad Alfiadh¹, Taufik Hidayat¹

¹Hasanuddin University, Faculty of Mathematics and Natural Sciences, Chemistry Department, Jl. Perintis Kemerdekaan, Tamalanrea Indah, Makassar City, South Sulawesi, Indonesia

²Hasanuddin University, Faculty of Agriculture, Jl. Perintis Kemerdekaan, Tamalanrea Indah, Makassar City, South Sulawesi, Indonesia

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¹³ Fall Armyworm (*Spodoptera frugiperda*) is one of the new pests from the Lepidoptera class, which causes huge losses for farmers, especially in corn plantations. The purpose of this study was to determine the effectiveness of cigarette butt waste and soursop leaf (*Annona muricata* L.) extracts as biopesticides against fall armyworms by observing the mortality and analyzing their LC₅₀. Extraction of cigarette butts waste and soursop leaves by ethanol 96% as solvent was carried out using Microwave-Assisted Extraction (MAE) method. As the comparison, the maceration and soxhletation extraction of soursop leaves were conducted and analyzed their chemical content by using LC-MS/MS instrument. The insecticide study used a completely randomized design (CRD) method to treat larvae instar III with cigarette butt waste and soursop leaf extracts with various concentrations in three repetitions. Data analysis was performed using probit analysis. The results showed that soursop leaf extract contained four major acetogenin compounds, namely muricatacin, annohexocin, robustocin, and epoxymurin, with a total percentage of 31.41% by using MAE method, which is higher than the acetogenin content of the maceration and soxhletation extracts (23.73% and 17.78%, respectively). Both cigarette butt waste and soursop leaf extracts were effective as biopesticides with an LC₅₀ value of 4.900% and 4.114%, respectively, in 12 hours of observation.

²¹ **Keywords:** *Annona muricata* L., cigarette butt, microwave-assisted extraction, natural insecticide, *Spodoptera frugiperda*

1 Introduction

⁴ *Spodoptera frugiperda* is a pest of maize that has been an inhabitant of the Central American continent (tropical climate) (Fan et al., 2020). This pest was reported to attack more than 200 plant species, including chilli, cabbage, rice, corn, tomatoes, beans, tobacco, eggplant, potatoes, peanuts, and soybeans (Montezano et al., 2018). Fall armyworm pests were reported to be spread in Japan, China, India, and various countries in Southeast Asia (Early et al., 2018). In 2019, *S. frugiperda* was first reported to attack several maize crops in Indonesia (Maharani et al., 2019).

⁴ FAW larvae can damage almost all parts of the corn plant (roots, leaves, male flowers, female flowers, and cobs) with a larval population of between 2–10 individuals per plant (Nonci et al., 2019). At the plant reproduction

stage, the attack rate of *S. frugiperda* was very high, with the percentage of infected plants at 49.2% (Deole & Paul, 2018). Its larvae were found on plant shoots. The plants are infected by this pest when the leaves have not fully opened (buds). If the leaves are open, many damaged leaf parts can be seen (Rwomushana, 2019). The damage in maize varieties was more significant than other plants, and the losses caused by this pest on maize in some countries were huge (Baudron et al., 2019) *Spodoptera frugiperda* J.E. Smith.

Fall armyworms have a very severe impact if not handled properly. Until now, there is no specific insecticide that can fight the pathogenicity impact of this pest (Firake et al., 2019). Farmers often control plant pests and diseases by using synthetic pesticides. However, it shows some negative impacts, which can interfere with human health

*Corresponding Author: Syadza Firdausiah, Hasanuddin University, Faculty of Mathematics and Natural Sciences, Chemistry Department, Jl. Perintis Kemerdekaan KM.10 Tamalanrea Indah, Makassar City, South Sulawesi, Indonesia. ORCID: <https://orcid.org/0000-0001-9420-9785>

and the surrounding ecosystem due to the carcinogenic properties (Hakeem et al., 2016). In addition, the presence of pesticide residues in the soil can poison non-target organisms, be carried to water sources, and poison the surrounding environment. The solutions to this problem must be sought immediately, one of which is by switching to safer biopesticides for the environment (Bateman et al., 2018).

Biopesticide is one alternative in pest and plant disease management (Rioba & Stevenson, 2020). Natural insecticides are defined as materials derived from living things (plants, animals, or microorganisms) that have the effect of inhibiting growth and development or killing pests or disease-causing organisms (Roopa et al., 2021). Biochemical biopesticide is one of the natural insecticides in which based on organic compounds of natural plants.

The plant that is commonly used as a biopesticide is tobacco (Villaverde et al., 2016). Tobacco is found in cigarette waste and contains various compounds that showed insecticidal activity (Millan et al., 2013). Cigarette butts contain the same content as tobacco, namely nicotine, phenol, and eugenol. Nicotine can be toxic to certain organisms, so that it can be used as a natural insecticide (Villaverde et al., 2016). In addition, cigarette butt extract showed good performance as bioinsecticide against *Anopheles stephensi* which caused malaria (Murugan et al., 2018), termites (Syifa et al., 2020), house fly larvae (*Musca domestica* L.) (Ahmed, 2018), and *Aedes aegypti* (Dieng et al., 2014).

Moreover, soursop plants (*Annona muricata* L.), particularly the leaves, contain a high level of acetogenin compounds (Coria-Teñez et al., 2018). This class of compounds performed insecticidal activity, which acts as mitochondrial poison (Isman & Seffrin, 2014) and caused death to the larvae and malformation to the surviving adult pests (Hidalgo et al., 2018). Some reports showed insecticidal activity of soursop leaf extract against *Tuta absoluta* (Ferrari de Brito et al., 2020), *Spodoptera litura* (Fathoni et al., 2013), *Aedes aegypti*, and other mosquito vectors (Santhosh et al., 2015).

This study aims to determine the effectiveness of cigarette butt waste and soursop leaves as biopesticides to control fall armyworms based on observations of mortality and LC₅₀ values using the Completely Randomized Design (CRD) method. The microwave-assisted extraction (MAE) method was used to get the cigarette butt and soursop leaf extracts due to the effectiveness and efficiency compared to other conventional techniques in the extraction process (Garrido et al., 2019). In our previous study, we compared three different extraction methods, namely maceration, soxhletation, and MAE, to extract cigarette butt waste. We found that the extract

resulted from MAE has the highest yield and nicotine content (Firdausiah et al., 2020). Thus, in this study, we also compared the yield and acetogenin content of these three extraction methods in the extraction of soursop leaves in ethanol 96%.

2 Material and methods

2.1 Sample preparation

Samples of cigarette butt waste (separated from unnecessary parts) and samples of fresh soursop leaves were prepared as much as 2 kg, cleaned with clean water, then air-dried. Next, the sample was mashed using a blender, then sieved using a 60 mesh sieve.

2.2 Extraction of samples

Twenty grams of each sample was extracted with 200 mL ethanol 96% in the microwave for 8 minutes at 100 W. The extract was filtered, and the residue was re-extracted twice. The residue was filtered out, and the solvent was evaporated to produce a thick extract. Then, it was dried in a desiccator until a dry extract was obtained (Firdausiah et al., 2020).

As the comparison, two other extraction methods, namely maceration and soxhletation, were performed for dried soursop leaf sample in ethanol 96% by following reference (Firdausiah et al., 2020). Controlling extraction time by TLC, the maceration extraction was occurred for 2 × 48 hours, while soxhlet extraction was performed for 12 hours.

2.3 Analysis of chemical content

Three extracts of soursop leaf from each extraction method were screened for their secondary metabolites content through phytochemical screening (alkaloids, flavonoids, terpenoids, steroids, saponins, and tannins) (Yadav et al., 2016) in the detection of the bioactive principles present in medicinal plants and subsequently may lead to drug discovery and development. In the present study, chief phytoconstituents of the six selected medicinal plants of different families were identified in order to relate their presence with bioactivities of the plants. Screening of six selected medicinal plants were performed for the presence of tannins, flavonoids, terpenoids, saponins, steroids, phlobatannins, carbohydrates, glycosides, coumarins, alkaloids, proteins, emodins and anthraquinones using standard methods. All the selected medicinal plants were found to contain Steroids and Coumarins except *Urginea indica* (Roxb.), and then analyzed for their chemical content using LC-MS/MS Shimadzu Mariner HP 5972.

2.4 Test larva rearing

S. frugiperda larvae were collected from corn plantations in Gowa district, South Sulawesi, Indonesia. The test larvae were taken randomly and fed until they entered the third star phase (Sakadzo et al., 2020). The rearing process was carried out at the Entomology/Plant Pest Laboratory, Faculty of Agriculture, Hasanuddin University.

2.5 Effectivity test of extracts as biopesticides

Preliminary tests were conducted to determine the range of concentrations of cigarette butt and soursop leaf extracts to be used in the study. This test was done to find a concentration range that could give the toxicity of the test larvae between 0% to 10%. Each of 10 test larvae was dripped by 0.5 mL of the extract solution. Then the larvae were put into a Petri dish and fed with baby corn. Mortality observations were carried out for 12 hours in 3 times of repetition. The preliminary test results were used to determine the concentration of the extract used for the toxicity test.

The solution of cigarette butt and soursop leaf extracts in ethanol 96% were made based on the concentrations obtained in the preliminary tests. Furthermore, 10 test larvae were dripped by each 0.5 mL of the extract solution to all parts of the body of the test larvae. Then the larvae were put into a petri dish and fed with baby corn. Mortality observations were carried out for 12 hours in 3 times of repetition.

3 Results and discussion

3.1 Extraction process of soursop leaf

The yield of microwave-assisted extract of soursop leaves was 12.9%, higher than the extract from the soxhletation method (12.06%) but lower than from the maceration method (14.64%). Based on the processing

time, the maceration method with a duration of 2×48 hours is longer than the MAE, which was 3×8 minutes, and soxhletation, which lasts for 12 hours. In this case, the longer the contact between the solvent and the extracted material, the greater the number of broken cells and the dissolved active ingredients (Wahyuni & Widjanarko, 2015). In addition, the maceration extraction method also has the advantage that it maintains the extracted active substance because it was carried out at room temperature (Azwanida, 2015) and other medicinal claims are now supported with sound scientific evidences. The study on medicinal plants started with extraction procedures that play a critical role to the extraction outcomes (e.g. yield and phytochemicals content). However, maceration is time-consuming extraction, which takes a longer time than soxhletation and MAE. Thus, it has limitations in terms of efficiency.

3.2 Chemical content of soursop leaf extracts

Phytochemical screening was carried out to overview the class of compounds contained in the soursop leaf extracts. The results of the phytochemical screening for each extract are given in Table 1. The results of the phytochemical screening test showed that soursop leaf extract from three extraction methods contained alkaloids, flavonoids, terpenoids, tannins, and polyketides. These results are in agreement with the report of Coria-Teilez et al. regarding the phytochemical content of soursop plants (Coria-Teilez et al., 2018).

Furthermore, the chemical content in each soursop leaf extract was analyzed using the LC-MS/MS instrument. The result showed that acetogenins are the major compounds in all extracts. There are four major acetogenin compounds contained in these extracts. The percentage of these compounds from soursop leaf extracts can be seen in Table 2.

Table 1 Phytochemical screening of soursop leaf extracts

Phytochemicals	Reagents	Extraction methods		
		maceration	soxhletation	MAE
Alkaloids	Dragendorf	+	+	+
	Mayer	+	+	+
	Wagner	+	+	+
Flavonoids	Mg + HCl	+	+	+
Terpenoids	Liebermann-Burchard	+	+	+
Saponins	H ₂ O	-	-	-
Tannins	FeCl ₃	+	+	+
Steroids	Liebermann-Burchard	-	-	-
Polyketides	Keddie	+	+	+

Table 2 Chemical percentage of acetogenin compounds in Soursop leaf extracts

Acetogenin compound (rt)	Extraction method		
	maceration (%)	soxhletation (%)	MAE (%)
Muricatacin (9.38 min)	8.19	7.42	12.75
Annohexocin (9.30 min)	8.28	6.75	8.32
Robustocin (10.03 min)	3.30	1.32	3.92
Epoxy-murin (10.38 min)	3.96	1.27	6.42
Total	23.73	17.78	31.41

These results are in accordance with the review article by Rady et al. (2018), which states the presence of these four chemical compounds in soursop plant extracts. The results also showed that the acetogenin content of the MAE extract was 31.41%, which is the highest percentage value compared to other extracts. The MAE method utilizes electromagnetic waves that can rupture the cell membrane of material and allow chemical constituents to be released into the solvent (Vinatoru et al., 2017). The advantage of the MAE method lies in the heating carried out in a closed system so that microwave energy can be propagated directly to the material through electromagnetic radiation (Garrido et al., 2019). In contrast, conventional extraction with heat transfer through conduction, convection and radiation methods allows a lot of heat energy to be lost to the environment. The energy that remains in the system will maximize the extraction process, minimizing the extraction time compared to the soxhletation method (Vinatoru et al.,

2017). The chemical structures of these acetogenin compounds are shown in figure 1.

3.3 Test larvae rearing

The test larvae used were *S. frugiperda* larvae instar III Phenotype 1 (F1). A total of 326 insect larvae were obtained from corn plantations in the districts of Gowa and Takalar. The rearing of larvae obtained from plantations to become pupae took approximately seven days, in which 62 pupae were obtained. The pupa phase to become an imago took three days. In this phase, 40 imagos were obtained. The imagos that successfully underwent fertilization produced eggs which then hatched into first instar larvae, in which at this stage, 521 larvae were obtained. Then the first instar larvae developed into third instar larvae, which took ten days, and 446 larvae were obtained. The larvae of the F1 Instar (III) test were then used in the preliminary test and mortality test. The rearing process of the test larvae can be seen in figure 2.

3.4 Preliminary test

This test was carried out to know the upper and lower threshold concentrations at which mortality of the test larvae occurred. Subsequently, a solution with various concentrations in the upper and lower threshold intervals was made for the toxicity test.

Both the mortality of *S. frugiperda* when treated by cigarette butt and soursop leaf extracts were dose-dependent, which the higher the concentration of the extract, the greater the percentage of mortality of the test larvae. It indicates that the concentration can affect the mortality of the test larvae. From Figure 3, the lower and upper threshold concentrations of cigarette butt extract were 2% and 10%, respectively. Moreover, the lower and upper threshold concentrations of soursop leaf extract were 2.5% and 6.5%, respectively, based on Figure 4. These results were used as the basis for the LC₅₀ toxicity test in 12 hours of observation.

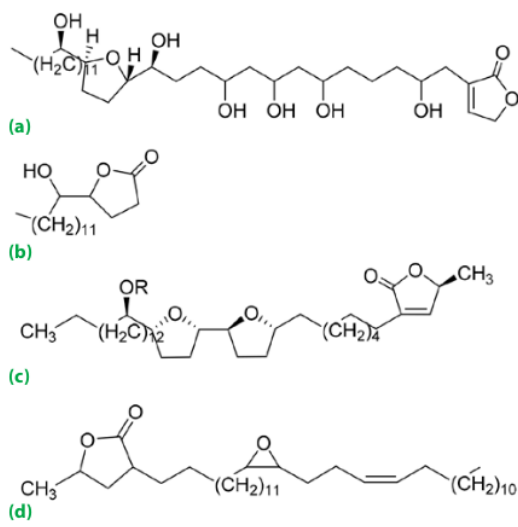


Figure 1 Chemical structures of annohexocin (a), muricatacin (b), Epoxy-murin (c), and robustocin (d)

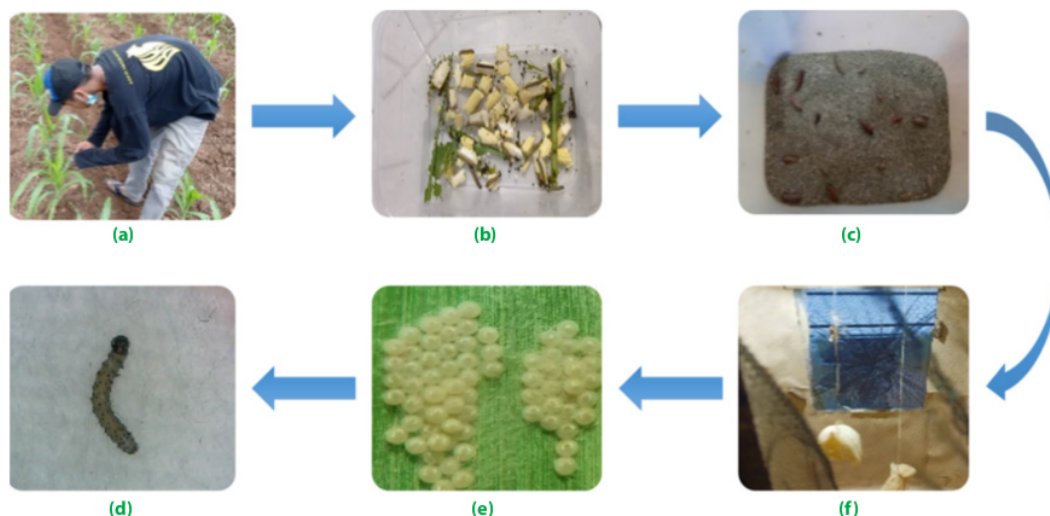


Figure 2 Test larvae preparation process: caterpillar collection on the field (a), rearing (b), pupae stage (c), imago stage (d), eggs production (e), phase III caterpillar (f)

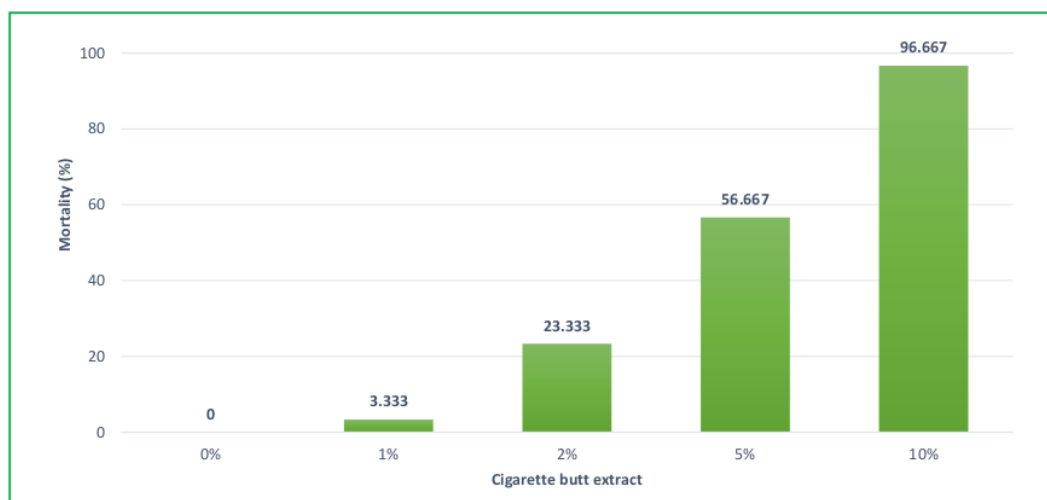


Figure 3 Mortality of *S. frugiperda* larva by the treatment of cigarette butt extracts

3.5 Toxicity test

A toxicity test is a follow-up test after the lower and upper threshold concentrations were obtained. Concentration variations were made for cigarette butt waste extract (2%; 4%; 6%; 8% and 10%) and soursop leaf extract (2.5%; 3.5%; 4.5%; 5.5%; and 6.5%) according to the results of the preliminary test. The toxicity test results of cigarette butt waste and soursop leaf extracts are shown in Table 3.

The LC_{50} values of cigarette butt waste and soursop leaf extracts were 4.900% and 4.114%, respectively, for 12 hours of observation. These findings showed better results than other reported biopesticides of *S. frugiperda*, such as aqueous extract of *Nicotiana tabacum* L., which showed 50% of effective concentration (Sakadzo et al., 2020), or moringa oil recommended to be used at 10% concentration (Kamel, 2010). However, both are still lower than some essential oils, i.e. *Ageratum conyzoides*, *Cymbopogon flexuosus*, and *Ocimum basilicum* (Rioba & Stevenson, 2020).

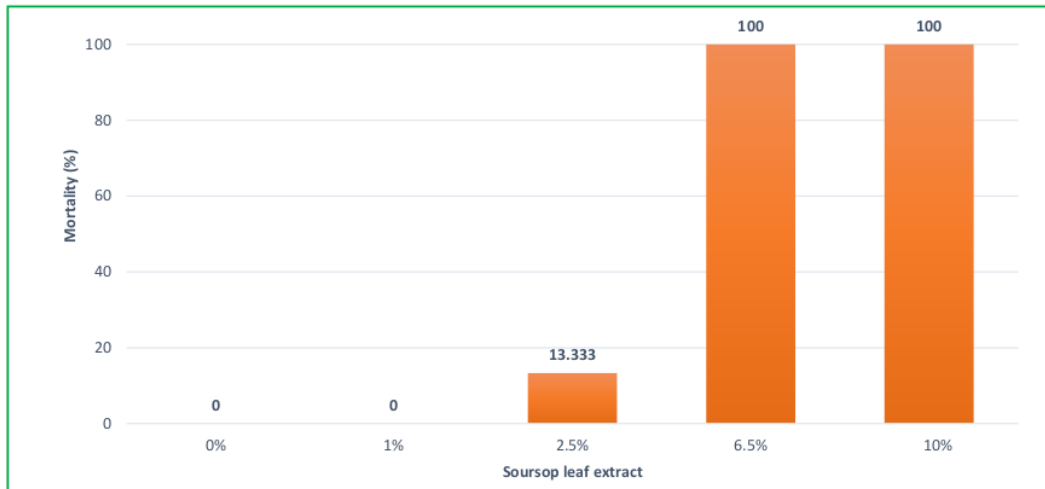


Figure 4 Mortality of *S. frugiperda* larva by the treatment of soursop leaf extracts

The mechanism of insecticides from cigarette butt waste extracts in poisoning armyworms is by attacking the nervous system. Nicotine contained in cigarette butt waste extract can attack the nervous system, especially the muscle nerves, which causes the nerves to become inactive and result in death (Shekins et al., 2016).

While in soursop leaf extract, acetogenin plays an important role. Acetogenin is toxic to mitochondria by inhibiting the process of energy production in these cells and will inhibit the formation of ATP (energy) in NADH. Acetogenin inhibits the enzyme ubiquinone oxidoreductase, an essential enzyme in complex I in the electron transport system, which ultimately causes oxidative phosphorylation in mitochondria. It disrupts the mechanism of nerve action that can cause death in pests (Hidalgo et al., 2018).

The morphological conditions of armyworms characterize the mechanism of action after treatment, which shows no movement, changes in skin color to dark, the body shrivels, stiffens, and secretes a brown liquid. The biopesticide extracts were directly impact the physical and metabolism of the test larvae so that they are effectively used as biopesticides by the direct exposure (contact) action method. **ther study should be performed in order to know the toxicity of both extracts to non-target organisms.**

4 Conclusions

MAE method for extracting soursop leaves were successfully extracted four main acetogenin compounds, namely muricatalicin, annohexocin, robustocin, and epoxymurin, in 31.41% from the extract, which better

Table 3 Larval mortality of *S. frugiperda* instar III

Cigarette butt extracts treatment (%)	Larval mortality (%)	Soursop leaf extract treatment (%)	Larval mortality (%)
0	0.000 ± 0.000 ^a	0	0.000 ± 0.000 ^a
2	23.333 ± 5.774 ^b	2.5	13.333 ± 5.774 ^b
4	53.333 ± 5.774 ^c	3.5	40.000 ± 40.000 ^c
6	66.667 ± 5.774 ^d	4.5	63.333 ± 5.774 ^d
8	86.667 ± 5.774 ^e	5.5	76.667 ± 5.774 ^e
10	96.667 ± 5.774 ^f	6.5	100.000 ± 0.000 ^f
F_{value}	149.120 (Sig = 0.000)	F_{value}	263.733 (Sig = 0.000)
LC ₅₀ (LCL-UCL)	4.900 (2.353–6.215)	LC ₅₀ (LCL-UCL)	4.114 (0.953–4.925)

Mean standard error followed by the same letter with in the column do not differ significantly ($P < 0.05$). LCL, lower confidence limit, UCL – upper confidence limit

than maceration and soxhletation extraction methods. Both cigarette butt waste extract and soursop leaf extract were effective as biopesticides against *S. frugiperda* with LC₅₀ values of 4.900% and 4.114%, respectively, for 12 hours of treatment.

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