



International Journal of Fisheries and Aquatic Studies

E-ISSN: 2347-5129

P-ISSN: 2394-0506

(ICV-Poland) Impact Value: 5.62

(GIF) Impact Factor: 0.549

IJFAS 2019; 7(6): 197-202

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www.fisheriesjournal.com

Received: 20-09-2019

Accepted: 24-10-2019

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Curcuma xanthorrhiza Roxb.: Control gyrodactyliasis in catfish *Clarias gariepinus*

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Abstract

This research based on the case of natural infection of *Gyrodactylus* sp in catfish (100-800 grams). The aim of the study was to control Gyrodactyliasis in catfish by using *Curcuma xanthorrhiza* Roxb. The catfish natural infected by *Gyrodactylus* sp macro-size type. The first step procedure to get the efficacy of a dose of *Curcuma xanthorrhiza* roxb against *Gyrodactylus* sp *in vitro*. The second was to observe the efficacy of *Curcuma xanthorrhiza* roxb dose *in vivo* which fish are naturally infected by *Gyrodactylus* sp. The third treatment was carried out on a field scale. The result showed that *Curcuma xanthorrhiza* roxb. was effectively to kill *Gyrodactylus* sp *in vitro* at a dose of 100 ppm in 446 minutes and 200 ppm for 200 minutes. While *Gyrodactylus* sp (without host) was able to live in water at 24 °C for 2880 minutes. The intensity of *Gyrodactylus* sp in chronic catfish was 1057 individuals per fish. While the highest fish mortality happened in case 4, it was 65.18% because found to be co-infected with pathogen bacteria. Bacterial infections greatly affect the mortality of catfish. The lowest fish mortality rate of 1.4% was in case 6. Immersion therapy for 24 hours using 100 ppm *Curcuma xanthorrhiza* Roxb. was effective to kill *Gyrodactylus* sp and it was safe for catfish. Treated fish will shorten the transmission of *Gyrodactylus* sp between fishes and reduce mortality. The use of *Curcuma xanthorrhiza* Roxb. can be recommended.

Keywords: *Curcuma xanthorrhiza* Roxb. *Gyrodactylus* sp, catfish, natural infection, therapy, bathing method

1. Introduction

Gyrodactyliasis is a disease caused by the worm *Gyrodactylus* spp. *Gyrodactylus* spp is one of platyhelminthes group parasite; monogenea. The breeding parasites by *viviparous* produces young worms and are also hermaphrodite which has male and female organs [3, 13, 21, 30]. One species of *Gyrodactylus* sp that raises problems in marine aquaculture is *Gyrodactylus salaris* which can infect salmon [5, 7, 10, 12, 16, 19, 21, 31].

Some cases of Gyrodactyliasis reported such as *Gyrodactylus alekosi* sp and *Gyrodactylus rysavyi* were identified from the North African catfish *Clarias gariepinus*. *Gyrodactylus rysavyi* was also reported from *Clarias gariepinus* from Kenyan and *Clarias anguillaris* from Senegal. *Gyrodactylus transvaalensis* and *Gyrodactylus gelnari* sp species were also identified from fish fin *Clarias anguillaris*, Senegal. While *Gyrodactylus turkanaensis* sp was identified from the gills of *Clarias gariepinus*, Kenyan [23]. While the Gyrodactyliasis outbreak caused by *Gyrodactylus jalalii* sp. identified from *Iranianocichla hormuzensis* fish [29].

Several results of the study were conducted to control Gyrodactyliasis disease. Use of acetic acid and sodium chloride 1: 2,000 by immersion. Use 5% salt soaking for 5 minutes and soaking in 1: 5,000 salt and formalin for 5-10 minutes [27]. Using 1% Virkon S disinfectant for 15 minutes, 80 mg / L Sodium percarbonate immersion 18 h [4]. 7.5 ppt ginger soaking for 90 minutes [11]. Woo and Leatherland (2011) state the use of Copper sulphate, formaldehyde, sodium chloride, hydrogen peroxide [25], dexamethasone [22] effective for the treatment of parasites. Therapy by manipulating the shock temperature of 40°C for 5-50 minutes [17].

The use of synthetic chemicals has been known to affect as a material for the prevention and control of health problems in aquaculture. The residue after the use of synthetic chemicals raises even greater problems. The use of the herb as a substitute is expected to be able to replace the function of chemicals that is quite safe for the continuity of aquaculture. It is known that some herbs can function as prevention and control of diseases caused by bacteria, parasites and fungi for aquaculture.

In fact, some herbals are considered to have immunostimulant functions and improve the fish appetite for fish [6].

The herbal used in this study were Java turmeric or *Curcuma xanthorrhiza* Roxb. in the local language called temulawak or Kuning Gedong. *Curcuma xanthorrhiza* Roxb. Also used as a natural antimicrobial to inhibit the growth of *Klebsiella pneumoniae* in food [26]. In addition, *Curcuma xanthorrhiza* Roxb. Also as an antioxidant, anti-inflammatory and anti-carcinogenic [1].

Curcuma (Zingiberaceae) is one of the large genera of rhizomatous and can be found in tropical countries such as Indonesia, India, Thailand, Malay islands, Northern Australia. In general, rhizomes are used in powder [15]. Elements contained in *Curcuma xanthorrhiza* Roxb. is Camphor; Zingiberene; γ -elemene; Trans β -farnesene; Ar-curcumene; Benzofuran; α -cedrene; β -elementsone and Xanthorrhizol. Besides that it also contains phytopharmaceuticals like terpenoids; phenols; flavonoids; saponins; cardiac glycoside; alkanoid and coumarin [1, 8, 9, 14, 18]. The aim of the study was to control Gyrodactyliasis in catfish by using *Curcuma xanthorrhiza* Roxb.

2. Materials and Method

2.1 Methodology

This test is based on the existence of natural infection of *Gyrodactylus* sp which infected catfish which are cultivated in ponds. In this case, three stages were carried out. The first step is the efficacy of several doses of *curcuma xanthorrhiza* roxb against *Gyrodactylus* sp (without fish / host) carried out in the laboratory. The second is the efficacy of the *curcuma xanthorrhiza* roxb dose test that fish which are naturally infected by the *Gyrodactylus* sp parasite is carried out in the laboratory. The third is treatment carried out on a field scale using *Curcuma xanthorrhiza* Roxb. Naturally infected catfish. Data collected in the form of the efficiency of several medicinal ingredients against *Gyrodactylus* sp. Dosage of *Curcuma xanthorrhiza* Roxb. for therapy of gyrodactyliasis. Efficacy of *Curcuma xanthorrhiza* Roxb. the Gyrodactyliasis was applied on a field scale. Prevalence, intensity and dominance of *Gyrodactylus* sp before and after therapy. As well as the clinical condition of fish infected *Gyrodactylus* sp.

2.2 Time and Place

Seven cases of gyrodactyliasis in catfish occurred in 2016 until 2017. Tests were carried out in the laboratory and the area of the MCFA Sukabumi, West Java, Indonesia.

2.3 Fish

Catfish from several cases of Gyrodactyliasis (Table 1).

Table 1: Case outbreak of *Gyrodactylus* sp in catfish (*Clarias gariepinus*)

Cases	Size (gram)	Number (fish)
1	300-800	6000
2	300-800	4000
3	300-800	4000
4	200-700	4500
5	100-200	3000
6	100-150	3000
7	300-400	3200

Gyrodactylus sp was taken by scraping from the surface of the body, fins, and gills. Observations were carried out microscopically using an Olympus microscope type BX53,

Stereo Zeiss type microscope and direct / visual observation. Calculating the prevalence, intensity and dominance of the *Gyrodactylus* sp parasite in each case [2, 23, 24].

2.4 *Curcuma xanthorrhiza* roxb

Curcuma xanthorrhiza Roxb. used dry powder. *Curcuma xanthorrhiza* roxb. obtained from the Spice and Medicinal Plant Research Center, Bogor, Indonesia.

2.5 The first step

This test aimed to prove the efficacy of *Curcuma xanthorrhiza* roxb. against of *Gyrodactylus* sp. The chemical used was potassium permanganate (5 ppm; 10 ppm; 25 ppm and 50 ppm), formalin (25 ppm and 100 ppm), *Curcuma xanthorrhiza* roxb (20 ppm; 100 ppm and 200 ppm) and as a control *Gyrodactylus* sp in water without added drug material. The chemicals were prepared in a glass beaker and filled with 30-35 live *Gyrodactylus* sp. Then calculate the time until the *Gyrodactylus* sp all died in each glass beaker.

2.6 The second step

This test aimed to determine the efficacy of *Curcuma xanthorrhiza* Roxb. on the survival of catfish. The catfish used were originated from case 1 (Table 2). Test containers used aquariums. The dose of *Curcuma xanthorrhiza* roxb used was 100 ppm and 200 ppm.

2.7 The three step

This test aims to determine the application of *Curcuma xanthorrhiza* Roxb. for the therapy of catfish which are infected *Gyrodactylus* sp which is cultivated in ponds and tanks. The fish used from cases 1 (table 3). Therapy is done in the pond. Therapy using a dose of 100ppm *Curcuma xanthorrhiza* Roxb. by 24-hour immersion.

3. Result

3.1 The first step

Table 2: The results of testing the efficacy of medicinal ingredients

Chemicals and Herb	Doses (ppm)	Time of survival <i>Gyrodactylus</i> sp (minutes)
Kalium Permanganate	5	354
	10	180
	25	165
	50	90
Formalin	20	1765
	100	204
	200	1620
<i>Curcuma xanthorrhiza</i> roxb	20	1620
	100	446
	200	200
Water (temperature 24 ⁰ C)		2880

Use of a 200 ppm dose of *Curcuma xanthorrhiza* Roxb. Can kill *Gyrodactylus* sp faster than 100 ppm. Although the application *Curcuma xanthorrhiza* Roxb. Was not as effective as potassium permanganate which can kill *Gyrodactylus* sp removed faster. But the dose of 200 ppm *Curcuma xanthorrhiza* roxb compared with 100 ppm formalin has the same effect of killing *Gyrodactylus* sp. From this first test, it can be concluded that *Curcuma xanthorrhiza* Roxb. Potential can be a drug therapy for Gyrodactyliasis in catfish. Therapy Gyrodactyliasis with *Curcuma xanthorrhiza* Roxb. Can use a dose of 100 ppm and 200 ppm.

3.2 The second step

The results of the identification of *Gyrodactylus* sp (in case 1) before treatment were prevalence 100%, the intensity of 46

individuals, the dominance of 79.2%. *Dactylogyrus* sp test results in prevalence 80%, intensity 5 individuals, dominant 42%. There was no bacterial infection in fish in this case 1.

Table 3: Catfish therapy using *Curcuma xanthorrhiza* roxb.

Therapy using <i>Curcuma xanthorrhiza</i> Roxb. (doses)	Identification result after therapy	The survival rate of fish after therapy
100ppm bathing for 24 h	Prevalensi <i>Gyrodactylus</i> sp 0%, Intensitas 0 individu	100%
100ppm bathing for 24 h	Prevalensi <i>Gyrodactylus</i> sp 0%, Intensitas 0 individu	75%
100ppm bathing for 24 h	Prevalensi <i>Gyrodactylus</i> sp 0%, Intensitas 0 individu	100%
200ppm bathing for 24 h	Prevalensi <i>Gyrodactylus</i> sp 0%, Intensitas 0 individu.	100%
200ppm bathing for 24 h	Prevalensi <i>Gyrodactylus</i> sp 0%, Intensitas 0 individu.	100%

The clinical condition of fish determined removed survival fish after therapy. Fish that died after therapy showed ulcers all of the body, crushed fish fins, runny fish mustaches and fish was anorexia before died. At the time of therapy, the fish looked very comfortable. Fish always stayed at the bottom of the container and done a little swimming.

The results of the identification of *Gyrodactylus* sp after therapy are negative. The dead *Gyrodactylus* sp will be

released from the body of the fish and can be easily seen in the water at the bottom of the aquarium. The dead *Gyrodactylus* sp looks milky white. Use of 100ppm and 200ppm *Curcuma xanthorrhiza* Roxb. safe for fish and does not cause fish to die.

3.3 The three-step

Table 4: Application of *Curcuma xanthorrhiza* roxb

Cases	Method	Therapy using <i>Curcuma xanthorrhiza</i> roxb. (hours)	Container	Implementation of treatment after it is known that fish are infected with <i>Gyrodactylus</i> sp (the day treatment after infected)
2	Bathing	24	Pond	22
3	Bathing	24	Tank	22
4	bathing 1	5	Pond	5
	bathing 2	24	Tank	7
5	Bathing	24	Tank	4
6	Bathing	24	Tank	2
7	Bathing	24	Tank	4

Table 5: The number of fish for the identification of parasite and mortality of fish.

Cases	Number of fish for the identification of parasite (fish)		Mortality of fish in ponds/tanks (%)
	Before therapy	After therapy	
2	5	50	17.78
3	3	50	26.68
4 Bathing 1	7	45	
4 Bathing 2		100	65.18
5	30	50	5.07
6	10	100	1.4
7	26	30	49.38

Table 6: The results of the identification of *Gyrodactylus* sp.

Cases	Before therapy			After therapy		
	Prevalence (%)	Intensity (individual)	Dominance (%)	Prevalence (%)	Intensity (individual)	Dominance (%)
2	100	54	83.5	0	0	0
3	100	197	100	0	0	0
4 Bathing 1	100	37	100	53.2	3	1.58
4 Bathing 2				0	0	0
5	100	13	100	0	0	0
6	100	21	100	0	0	0
7	100	9	100	0	0	0

In each case, the rates of *Gyrodactylus* sp infection differed in fish (Table 6). Low mortality rates in fish are caused by a single infection of *Gyrodactylus* sp. Whereas, fish with a high mortality rate are also affected by another micro-organism as co-infection. Like in cases 3, 4 and 7, bacteria become co-infections in these fish.

The therapy was carried out at different times after it was known that fish are infected with parasites (Table 4). Although the implementation of therapy was carried out on the 22nd day after fish infected with *Gyrodactylus* sp can still

result in a low mortality rate (cases 2). This is because fish are only infected with a single pathogen, *Gyrodactylus* sp. Whereas in cases 5 and 7 and also cases 2 and 3, the mortality rate was different in fish even though the implementation of therapy was carried out at the same time (Table 5). This is affected by bacterial infections. Therapy that was done faster after the fish are known to be infected, it will reduce the mortality rate in fish.

In case 4, therapy was done twice. The first therapy was done in the pond. After the first post-treatment examination,

Gyrodactylus sp was found, although the prevalence and intensity values decreased. This happened because the dose estimation was not appropriate and also the uneven shape of the pond construction so that the calculation of the water

volume became incorrect that it will affect the dose. For the effectiveness of drug administration, the second therapy that was carried out in the tank it was necessary and gave *Gyrodactylus* sp negative.

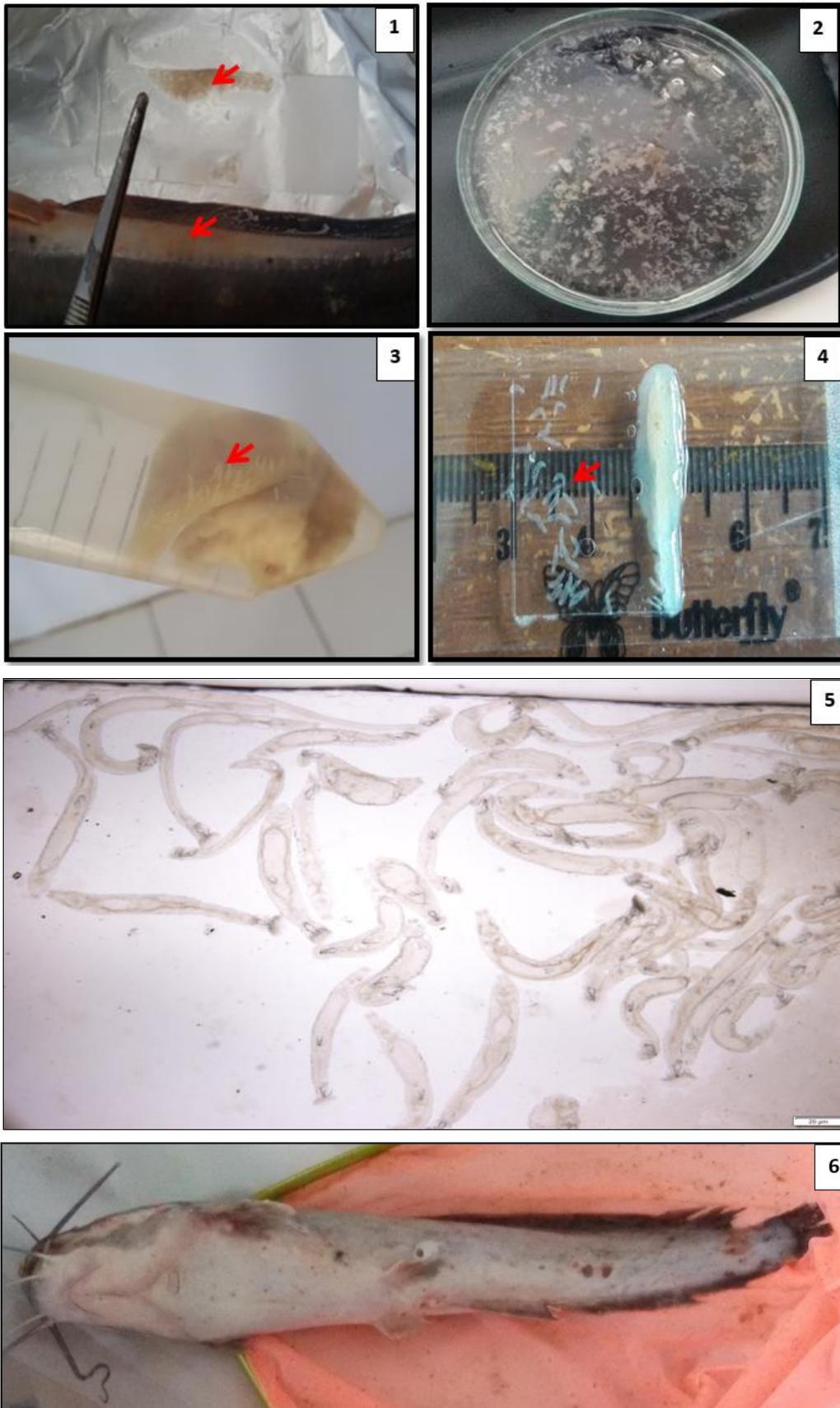


Fig 1-6: (1) *Gyrodactylus* sp collected from catfish bodies (2) *Gyrodactylus* sp (3) *Gyrodactylus* sp which was still attached to pieces of stomach meat of catfish stored in formalin solution (4) *Gyrodactylus* sp preparation for observation under a microscope (5) *Gyrodactylus* sp under a 40x microscope (6) the condition of catfish infected by *Gyrodactylus* sp.

The number of *Gyrodactylus* sp collected from one fish of acutely-infected catfish can reach 1057 individuals (Figure 1). The condition of the fish was very weak and quickly died when lifted out of the water. The fish always swim around the pool inlet. *Gyrodactylus* sp was very easily seen directly with the naked eye (Figure 1, Figure 2, Figure 3 and Figure 4). The macro size of *Gyrodactylus* sp made it easier to examine the field. *Gyrodactylus* sp becomes milky white when it was dead (Figure 2, Figure 3, Figure 4). The condition of catfish infected with *Gyrodactylus* sp had excessive mucus secretion and the color of the body of the fish became pale. In severe *Gyrodactylus* sp infections showed the fish fin was damaged (erosion), especially on the dorsal fin, caudal fin, abdominal fins and body parts to be injured and damage to the mustache (Figure 6).

Curcuma xanthorrhiza roxb powder. spread immediately on water. After 24 hours, water was replaced by new freshwater. Catfish can survive even though they was soaked in 100 ppm *Curcuma xanthorrhiza* Roxb. for 2 days without water replacement. *Curcuma xanthorrhiza* roxb. Therapy were very easy and effective, it did not cause discoloration, loss of appetite and death in fish.

4. Discussion

The condition of catfish infected with *Gyrodactylus* sp (Figure 6) was in accordance with the statement of [20, 21]. The condition of fish fin and mustache erupted, the color of the body of the fish became pale, the fish excessive mucus secretion so that the body of the fish felt visible. Abnormal behavior in fish infected with *Gyrodactylus* sp, namely; swim on the surface of the water, at the edge of the pool, always swim in the inlet and suddenly swim very quickly into the body of water. This showed that the fish feels uncomfortable, itchy, painful due to the large number of *Gyrodactylus* sp in its body so they tried to remove *Gyrodactylus* sp from its body [21]. Reported that salmon mortality could reach 100% in *Gyrodactylus salaris* infection if no treatment was taken. In this study, seven cases of natural infection with *Gyrodactylus* sp in catfish. The percentage of mortality was high in fish case 3, 4 and case 7. This was due to the role of bacterial infection. The intensity of *Gyrodactylus* sp in fish in acute conditions was 1057 individuals. The fish can still survive and swim slowly around the pool inlet. This condition was carried out in order to get enough oxygen to survive. According to [30], the intensity of *Gyrodactylus* sp infection can reach 600 individuals per fish.

Therapy using 100ppm *Curcuma xanthorrhiza* roxb by immersion for 24 hours can shed *Gyrodactylus* sp in catfish size 100-800 grams (Table 4). Although the results of the 100ppm *Curcuma xanthorrhiza* roxb dose efficacy test can kill *Gyrodactylus* sp in 446 minutes (Table 2). But for applications in the field it takes longer to take into account the number of fish treated. Immersion therapy was done for the treatment of Gyrodactyliasis because the parasite infected the outside of the fish's body [32]. Calculation of water volume was an important factor for the success of treatment with immersion methods. Some factors that need to be considered for the application of treatment include water quality, fish conditions, economy and safety of the environment and several other factors such as the results of parasite type identification, chemical selection, strategy and dose accuracy [28].

Fish that have been known to be infected with parasites should be treated immediately. Immediate treatment can

reduce fish mortality and narrow the area of transmission of parasitic infections. Moreover, the transmission and infection of *Gyrodactylus* sp are very fast. Considering the breeding of *Gyrodactylus* sp by giving birth so that *Gyrodactylus* sp's puppies are ready to infect other fish. As in case 6, the time for therapy is done faster than the time of infection so that it can reduce the percentage of fish mortality (Table 5).

The conclusion in this study that *Curcuma xanthorrhiza* Roxb proven to be effective and easy to apply for the treatment of *Gyrodactylus* sp in catfish. *Curcuma xanthorrhiza* Roxb can be a safe alternative therapy than chemicals that have negative effects on the environment and humans. Moreover, waste and residues from this therapy are safe compared with chemicals.

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