Available Online at EScience Press
Plant Protection
ISSN: 2617-1287 (Online), 2617-1279 (Print)
http://esciencepress.net/journals/PP

COMPARATIVE EFFICACY OF SYNTHETIC INSECTICIDES AGAINST CITRUS PSYLLA, *DIAPHORINA CITRI* KUWAYAMA (HOMOPTERA: PSYLLIDAE) UNDER LABORATORY AND FIELD CONDITIONS

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ARTICLE INFO

Article history
Received: 1st March, 2022
Revised: 4th April, 2022
Accepted: 6th April, 2022

Keywords
Diaphorina citri
Politrin-C®
Confidor®
Imazone®
Talstar®

ABSTRACT

Citrus fruits are rich in macronutrients, such as simple sugars and nutritional fiber, and provide many kinds of micronutrients containing thiamine, vitamin B6, riboflavin, pantothenic acid, potassium, calcium, phosphorus, magnesium, and copper, which are vital for keeping health and normal growth. Production of citrus was strongly affected by citrus psylla (*Diaphorina citri)*. The present study was carried out to determine the effectiveness of some synthetic insecticides with different concentrations against citrus psylla under field and laboratory conditions. Four insecticides viz. Politrin-C® (Profenofos + Cypermethrin), Talstar® (Bifenthrin), Confidor® (Imidacloprid), and Cymbush® (Cypermethrin) were used for evaluation of their effectiveness against citrus psylla. For laboratory experimentation, four concentrations of each insecticide along with one control treatment were made. For the field experiment, all the said insecticides were applied at field recommended doses. Citrus psylla was collected from an infested field of citrus crop and transferred in a plastic cage (60×60×60 cm) and kept in the IPM laboratory under standard laboratory conditions in the Department of Entomology, University of Agriculture Faisalabad Pakistan. Fresh leaves of citrus were used for the insect rearing as a diet for the insects. The rearing conditions were maintained at 25±2°C and 60-70% RH. Results revealed that LC_{50} values were 3.23%, 5.60%, 5.42% and 3.09% after 6 hours, 0.41%, 1.41%, 2.42% and 1.90% after 12 hours and 0.08%, 0.09%, 0.49% and 1.80% for Politrin-C®, Talstar®, Confidor® and Imazone® after 24 hours respectively. Politrin-C® was found to be more effective because of causing maximum mortality (71.15%), (51.20%), and (34.64%) after 24 hours, 12 hours, and 6 hours respectively of post-treatment application. The maximum population reduction of 66.68% was recorded with Politrin-C® followed by 62.96%, 61.23%, and 59.68% population reductions observed with treatments with Confidor®, Imazone®, and Talstar® respectively. However, Politrin-C® proved to be more toxic and considered effective for *D. citri*.

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INTRODUCTION

Asian citrus psylla (ACP), *Diaphorina citri* Kuwayama (Homoptera: Psyllidae) is a major pest of citrus and was first described in Tiawan . It is widely distributed in

many areas of Pakistan, India and China. ACP is a vector of fatal citrus disease called 'Huanglongbing' (HLB) or citrus greening (Bové, 2006; Halbert and Manjunath, 2004; Pluke et al., 2008; Su et al., 1991). HLB is the most distractive disease of citrus and is currently present in all the citrus growing areas of the world (Halbert and Manjunath, 2004; Manjunath et al., 2008). It infects orchard which is completely destroyed in 6 to 7 years (Aubert, 1990; Bové, 2006) and infected tress shows stunting, off-season bloom, premature fruit drop and small, misshapen, bitter fruit (Bové, 2006; Halbert and Manjunath, 2004). The population of citrus psylla fluctuates in relation to temperature and relative humidity. Citrus psylla peaks twice a year, which coincides with the periods of citrus flushing in the spring and summer (Sahu and Mandal, 1997; Wang, 2002).

About 25% yield loss is caused by the insect pests in citrus and 83-95 percent of that 25% loss is caused by *D. citri* (Shivankar and Singh, 2006). In Florida, the losses due to invasion of ACP and its vectored disease huanglongbing is about 10 percent resulting in about 8257 job losses, revenue reduction of $ 2.7 billion (US) and $ 1.8 billion (US) economic activities related to citrus work force (Hodges and Spreen, 2012).

In Pakistan, control of insect pest is usually done by the application insecticides as cover spray (Qureshi et al., 2013; Rogers, 2008; Sétamou et al., 2010; Srinivasan et al., 2008). Numerous synthetic insecticides incude (Dimethoate, monocrotophos, phosphamidon, confidor, decamethrin and fenvalevate), botanicals (neem oil, spray oils (petroleum) and insect growth regulators were tried against citrus psylla with encouraging results based on which recommendations have been made. Two to three sprays at 10-15 days interval were found to be effective against citrus psylla (Qureshi et al., 2013; Qureshi et al., 2012; Qureshi et al., 2011; Qureshi and Stansly, 2007; Qureshi and Stansly, 2008; Qureshi and Stansly, 2009, 2010; Qureshi et al., 2009). Different methods include foliar application of systemic insecticides incuding imidacloprid, thiamethoxam and clothianidin proved to be effective against ACP for the reduction about 50-70 percent population in pick seasons and maximum mortality incudes 70 to 80 percent of ACP was achieved by the application pyrethroids insecticides (Hussain and Siddique, 2010; Ichinose et al., 2010; Serikawa et al., 2012).

As ACP and HLB pose a serious threat to citrus production in Pakistan and conventional control methods are unable to control this factor, therefore, the present study was carried out to evaluate the effectiveness of insecticides against citrus psylla.

MATERIALS AND METHODS

Rearing of Asian citrus psyllid

The psyllid colony was maintained on *Murraya paniculata* (Citrus Jasmine) plants in controlled temperature glass house at 25 ± 2°C and 60-70%RH at 16L: 8D in IPM laboratory, Department of Entomology, University of Agriculture, Faisalabad-Pakistan. Adults of *Diaphorina citri* were collected from nearby citrus orchards by using aspirators. Rearing of psyllid were done in plastic cage (60×60×60 cm) kept in IPM laboratory. Fresh leaves of citrus were used for the insect rearing as diet of the insects.

Laboratory study (leaf dip bioassay)

Bioassays of different insecticides (Politrin-C® (Profenofos + Cypermethrin), Talstar® (Bifenthrin), Confidor® (Imidacloprid) and Cymbush® (Cypermethrin) were performed by using leaf dip method. A stock solution (D-1) of the highest concentration was prepared for each insecticide and the serial dilutions were made by taking half of the stock solution and diluting it with distilled water to the original volume in another measuring cylinder to make D-2. Successive dilutions were made until the last dilution for each of the insecticide is achieved (Table 1).

Table 1: Insecticides and their concentrations used in the study.

Concentration (ppm)	Insecticides			
	Politrin-C®	Bifenthrin®	Imidacloprid®	Cypermethrin®
C1	1	0.8	1	1
C2	0.5	0.4	0.5	0.5
C3	0.25	0.2	0.25	0.25
C4	0.125	0.1	0.125	0.125
C0	-	-	-	-

For each insecticide concentration, at least 15 adult Asian citrus psyllids (ACP) were treated with four replicates and each insecticide was tested at four concentrations. Fresh leaves were cut as disc sized and dipped in each concentration for 30 s and allowed to air dry at room temperature for half an hour. After drying, the treated leaves were kept in Petri dishes. Moist filter paper was used in each petri dish to prevent the desiccation of leaf disc. For the control treatment, leaf disc was dipped in water alone. For bioassay study, 15 psyllid adults were placed in 60 mm plastic disposable petri dishes with 60 mm citrus leaf disc placed over agar beds as food. Petri dishes with insects were kept at 25± 1 ◦C and 50 ± 5% RH with 12 hours light in controlled condition. Mortality data of psyllids were recorded after 6 hrs, 12 hrs and 24 hrs. After treatments, adult psyllids were disturbed by soft camel hair brush to confirm whether they are alive or dead. ACP adults that did not move upon prodding were considered dead. The collected data were subjected to Probit analysis after converting the data into percent corrected mortality as described by Henderson and Tilton (1995) given below to determine LC_{50}.

$$\text{Corrected } \% = \left(1 - \frac{\text{n in C before treatment} \times \text{n in T after treatment}}{\text{n in C after treatment} \times \text{n in T before treatment}}\right) \times 100$$

Where: n = Insect population, T = treated, C = control

Field study

For field study, fifteen citrus trees (sour orange variety) of equal size were selected representing five treatments with three replications. Each plant was represented an experimental unit. On each plant, 4 branches, one on each side, were randomly selected and tagged. The insecticides were sprayed at each selected plant on the bases of recommended dose early in the morning, whereas, the control plants were left untreated. The populations of citrus psyllid were counted on 20 leaves of each branch (80 leaves/tree). The data were recorded after 24 hours, 48 hours and 72 hours of application of insecticides. Percent population change was calculated by using modified Abbot's formula (Abbott, 1925).

$$\text{Percent population change} = 1 - \frac{P_oP_t}{P_rP_t} \times \frac{P_rP_c}{P_oP_t} \; X \; 100$$

P_oP_i = Population after treatments

P_rP_t = Population before treatment

P_rP_c = Population in the control before spray

P_uP_c = Population in the control after spray

Statistical analysis

The mortality data were converted into percentage corrected mortality by using Abbot's formula (Abbott, 1925). Data obtained in various treatments of different concentrations were compared by ANOVA and all means were compared by using Tukey's HSD test. For the analysis of data Statistica 8.1 software was used. Field data on percentage population reduction were compared by ANOVA and all means were compared by using LSD test.

RESULTS

Toxicity of insecticides against *Diaphorina citri* at different post treatment intervals under laboratory conditions

The toxicity of insecticides evaluated against *D. citri* varied significantly when their releases were made at 6, 12 and 24 hours post treatment interval at 95% fiducial limits (Table 2). When LC_{50} values of the insecticides were compared with their respective field recommended dose rates after 6 hours, it was found that Politrin-C®, Talstar®, Confidor® and Cymbush® had 12.92, 28, 13.55 and 12 times respectively higher than field recommended doses. After 12 hours of post treatment intervals, LC_{50} values of 1.64, 7.05, 6.05 and 7.6 times respectively were higher than field recommended doses. After 24 hours, LC_{50} values for Politrin-C® and Talstar® were 3.2 and 2.22 times lower than field recommended doses while Confidor® and Cymbush® had 1.23 and 4.32 time higher than field recommended doses. These results indicated that Politrin-C® and Talstar® were more toxic after 24 hours intervals while Confidor® and Cymbush® were least toxic against citrus psylla. After 24 hours post treatment, ACP was found to be susceptible to Politrin-C® and Talstar® while for Confidor® and Cymbush® it showed resistance.

Maximum mortality of 71.15% was observed after 24 hours post treatment exposure for Politrin-C® followed by 65.05% for Talstar®, 53.40% for Confidor® and 45.10% for Cymbush®. After 12 hours post treatment exposure, mortality of 51.20% observed for Politrin--C® (Profenofos + Cypermethrin) followed by 41.20% for Talstar® (Bifenthrin), 35.45% for Confidor® (Imidacloprid) and 38.45% for Cymbush® (Cypermethrin). After 6 hours post treatment exposure, the maximum mortality of 34.65% was observed for Politrin-C® followed by 30.05% for Talstar®, (3.80% for Confidor® and 30.60% for Cymbush®.

Table 2: LC_{50} values of different concentrations of some pyrethroids insecticides for citrus psylla, *Diaphorina citri* Kuwayama at C.I. 95 % after different time intervals under laboratory conditions.

Time	Pesticide (active ingredient)	FRD	χ^2 (df)	LC_{50}	95% FL	Slope ± SE
6 Hours	Politrin-C® (Profenofos + Cypermethrin)	2.5 ml/L	0.08 (2)	32.3 ml/L	2.01-10.93	0.34 ± 0.11
	Talstar® (Bifenthrin)	2 ml/L	2.24 (2)	56 ml/L	2.99-60.04	0.33 ± 0.11
	Confidor® (Imidachloprid 20 SL)	2.5 ml/L	0.23 (2)	54.2 ml/L	3.57-13.26	0.59 ± 0.13
	Cymbush® (Cypermethrin)	2.5 ml/L	1.51 (2)	30 ml/L	2.14-6.16	0.45 ± 0.11
12 Hours	Politrin-C® (Profenofos + Cypermethrin)	2.5 ml/L	0.01 (2)	4.1 ml/L	0.05-0.76	0.27 ±0.09
	Talstar® (Bifenthrin)	2 ml/L	1.49 (2)	14.1 ml/L	0.59-2.77	0.26 ± 0.09
	Confidor® (Imidachloprid 20 SL)	2.5 ml/L	0.27 (2)	24.2 ml/L	1.71-4.18	0.44 ± 0.10
	Cymbush® (Cypermethrin)	2.5 ml/L	1.20 (2)	19.00 ml/L	1.30-3.01	0.40 ± 0.09
24 Hours	Politrin-C® (Profenofos + Cypermethrin)	2.5 ml/L	0.39 (2)	0.8 ml/L	0.00-0.21	0.39 ± 0.09
	Talstar® (Bifenthrin)	2 ml/L	1.65 (2)	0.9 ml/L	0.01-0.24	0.29 ± 0.08
	Confidor® (Imidachloprid 20 SL)	2.5 ml/L	0.77 (2)	4.9 ml/L	0.16-0.77	0.37 ± 0.09
	Cymbush® (Cypermethrin)	2.5 ml/L	0.78 (2)	10.8 ml/L	0.58-1.57	0.36 ± 0.09

FRD = Field recommended dose; χ^2 = Chi square value; df = Degree of freedom LC = Lethal Concentration; FL = Fudicial Limit; SE = Standard error

As Figure 1 Illustrated that, the maximum mortality was observed in population treated with Politrin-C® followed by Talstar®, Confidor® and Cymbush® after different time intervals (Table 3).

Maximum mortality of 74.08% for Politrin-C®was observed at the highest concentration (C_1) as compared to control (7.66%) which indicated that at highest concentration Politrin-C® was 11.12 time more toxic than water application to citrus psylla. Mortality about 64.16% for Talstar® as compared to control 6.83% indicated that Talstar® was 9.39 time more toxic to citrus psylla, 61.33% for Confidor® as compared to control 6.75% indicated that Confidor® was 9.08 time more toxic than water application to citrus psylla and mortality of 59.25% for Cymbush® as compared to control 6.91% indicated that Cymbush® was 8.57 time more toxic than water application to citrus psylla. LC_{50} values indicated that Politrin-C® was 10 times more toxic than Talstar® and Talstar® was 3 times more toxic than Confidor® and Confidor® was 3 times more toxic than Cymbush® to citrus psylla. Maximum mortality of 67.08% was observed at concentration of 2 ppm for Politrin-C® (Profenofos + Cypermethrin), 56.83% for Talstar® (Bifenthrin) 47.00% for Confidor® (Imidacloprid) and 52.25% for Cymbush® (Cypermethrin). Maximum mortality was observed at concentration of 1 ppm which was 60.08% for Politrin-C®, 56.83% for Talstar® 40.00% for Confidor® and 45.25% for Cymbush®. Maximum mortality observed at concentration of 0.5 ppm was 52.75% for Politrin-C®, 42.50% for Talstar® 32.66% for Confidor® and 30.58% for Cymbush® (Table 4).

Table 3. Means of percentage mortality of citrus psylla, *Diaphorina citri* Kuwayama at various time intervals for different insecticides under laboratory conditions.

Times Intervals	Insecticides ± S.E.			
	Politrin-C® (Profenofos + Cypermethrin)	Talstar® (Bifenthrin)	Confidor® Imidacloprid	Cymbush® Cypermethrin
24 hours	71.15 ± 0.91^{A}	65.05 ± 0.12^{A}	53.40 ± 0.09^{A}	45.10 ± 0.18^{A}
12 hours	51.20 ± 0.45^{B}	41.20 ± 0.08^{B}	35.45 ± 0.04^{B}	33.85 ± 0.13^{B}
6 hours	34.65 ± 0.88^{C}	30.05 ± 0.01^{C}	26.80 ± 0.02^{C}	22.60 ± 0.11^{C}

Table 4. Means percentage mortality of citrus psylla, *Diaphorina citri* Kuwayama at different concentrations for various insecticides under laboratory conditions.

Concentrations (ppm)	Insecticides ± S.E.			
	Politrin-C® (Profenofos + Cypermethrin)	Talstar® (Bifenthrin)	Confidor® (Imidacloprid)	Cymbush® (Cypermethrin)
C_1	74.08 ± 1.87^{A}	64.16 ± 1.64^{A}	61.33 ± 1.31^{A}	59.25 ± 1.22^{A}
C_2	67.08 ± 1.43^{B}	56.83 ± 1.31^{B}	54.00 ± 1.16^{AB}	42.25 ± 1.10^{B}
C_3	60.08 ± 1.22^{BC}	48.83 ± 1.22^{BC}	46.00 ± 1.26^{B}	38.25 ± 1.08^{BC}
C_4	52.75 ± 1.54^{C}	$42.50^{C} \pm 1.41^{C}$	32.66 ± 1.33^{C}	30.58 ± 1.03^{C}
Control	7.66 ± 1.01^{D}	6.83 ± 1.54^{D}	6.75 ± 1.21^{D}	6.91 ± 1.01^{D}

Field Study

After 1st spray, after 24 hours of post treatment intervals, the maximum population reduction of 16.19% was observed in Politrin-C® (Profenofos + Cypermethrin) followed by Confidor® (Imidacloprid) (9.74%), Cymbush® (Cypermethrin) (8.57%) and Talstar® (Bifenthrin) (7.55%) while the minimum population reduction was observed in control treatment (2.68%). After 48 hours, the maximum population reductions of 47.25%, 42.85%, 42.85%, 39.65% and 2.66% were observed for Politrin-C® (Profenofos + Cypermethrin) followed by Confidor® (Imidacloprid), Cymbush® (Cypermethrin), Talstar® (Bifenthrin) and control treatment respectively (Table 5).

Table 5. Means of population reduction percentage of citrus psylla, *Diaphorina citri* Kuwayama for different insecticides at C.I. 95 % after 24 hours under filed conditions after 1st and 2nd spray.

Treatments	Population Reduction Percentage ± S.E.					
	After 1st spray			After 2nd spray		
	24 hours	48 hours	72 hours	24 hours	48 hours	72 hours
Politrin-C® (Profenofos + Cypermethrin)	$16.19^{A} \pm 0.67$	$47.25^{A} \pm 0.81$	$66.68^{A} \pm 1.51$	$32.38^{A} \pm 0.67$	$74.51^{A} \pm 0.83$	$87.17^{A} \pm 1.59$
Confidor® (Imidacloprid)	$9.74^{B} \pm 0.62$	$42.85^{B} \pm 0.42$	$62.96^{B} \pm 0.97$	$19.49^{B} \pm 0.71$	$65.71^{B} \pm 0.51$	$81.71^{B} \pm 1.21$
Cymbush® (Cypermethrin)	$8.57^{BC} \pm 0.78$	$42.85^{B} \pm 0.75$	$61.23^{BC} \pm 1.02$	$17.14^{BC} \pm 0.54$	$55.71^{B} \pm 0.67$	$73.71^{C} \pm 1.34$
Talstar® (Bifenthrin)	$7.55^{C} \pm 0.64$	$39.04^{C} \pm 0.94$	$59.68^{C} \pm 1.01$	$15.11^{C} \pm 0.39$	$48.10^{C} \pm 0.81$	$66.10^{B} \pm 0.91$
Control	$2.68^{D} \pm 0.91$	$2.66^{D} \pm 0.21$	$4.00^{D} \pm 2.01$	$5.36^{D} \pm 0.27$	$6.33^{D} \pm 0.34$	$10.33^{D} \pm 0.74$

Values in same column containing different letters of same format are significantly different from each other at probability level of 5%.

Similarly after 72 hours the maximum population reduction of 66.66%, 62.96%, 61.23%, 59.68% and 4.00% were observed for Politrin-C® (Profenofos + Cypermethrin) followed by Confidor® (Imidacloprid), Cymbush® (Cypermethrin), Talstar® (Bifenthrin) and control treatment respectively. After 2nd spray, after 24 hours of post treatment intervals the maximum population reduction (32.38%) was observed in Politrin-C® (Profenofos + Cypermethrin) followed by Confidor® (Imidacloprid) (19.49%), Cymbush® (Cypermethrin) (17.14%) and Talstar® (Bifenthrin) (15.11%) while the minimum population reduction was observed in control treatment (5.36%) while after 48 hours maximum population reduction 74.51%, 65.71%, 55.71%, 48.10% and 6.33% were observed for Politrin-C® (Profenofos + Cypermethrin) followed by Confidor® (Imidacloprid), Cymbush® (Cypermethrin), Talstar® (Bifenthrin) and control treatment respectively. Similarly, after 72 hours maximum population reduction 87.17%, 81.71%, 73.71%, 66.16% and 10.33% were observed for Politrin-C® (Profenofos + Cypermethrin) followed by Confidor® (Imidacloprid), Cymbush® (Cypermethrin), Talstar® (Bifenthrin) and control treatment respectively (Table 5).

DISCUSSION

Present study was carried out to evaluate the susceptibility level of ACP to conventionally used pyrethroids insecticides and verified reduced susceptibility to several insecticides among laboratory stains of ACP. After 6 hours post treatment application, LC_{50} values were 3.23%, 5.60%, 5.42% and 3.09% for Politrin-C® (Profenofos + Cypermethrin), Talstar® (Bifenthrin), Confidor® (Imidacloprid) and Cymbush® (Cypermethrin) respectively which was 12-28 times higher than filed recommended doses. Talstar® showed the highest LC_{50} followed by Confidor®, Politrin-C® and Cymbush® which indicated that Cymbush® was more toxic than Confidor® after 6 hours of post treatment intervals. The Probit analysis indicates (LC_{50}> FRD) for Politrin-C® (Profenofos + Cypermethrin), Talstar® (Bifenthrin), Confidor® (Imidacloprid) and Cymbush® (Cypermethrin) after 6 hours of treatment against citrus psylla showed that at short time period these insecticides are least toxic.

These results are in line with those obtained by Kim et al. (2006) who tested that after 6 hours of exposure of methoxyfenozide, Bifenthrin and Cypermethrin there had no sub lethal effects on adults of plant bug *Deraeocoris brevis*. After 12 hours of post treatment application LC_{50} values were 0.41%, 1.41%, 2.42% and 1.90% for Politrin-C® (Profenofos + Cypermethrin), Talstar® (Bifenthrin), Confidor® (Imidacloprid) and Cymbush® (Cypermethrin) respectively. The Probit analysis indicates (LC_{50}> FRD) for Politrin-C® (Profenofos + Cypermethrin), Talstar® (Bifenthrin), Confidor® (Imidacloprid) and Cymbush® (Cypermethrin) after 12 hours of treatment against citrus psylla indicated that theses pesticides are least toxic and unable to kill 50% population. The results are in line with those obtained by Ambrose and Lee (2003) who tested that there were no harmful effects of methoxyfenozide on numbers of useful or pest species population density, Kim et al. (2006) who tested that at the full rate of methoxyfenozide, there had no sub lethal effects on adults of plant bug *Deraeocoris brevis*. After 24 hours post treatment intervals, LC_{50} values were 0.08%, 0.09%, 0.49% and 1.80% for Politrin-C® (Profenofos + Cypermethrin), Talstar® (Bifenthrin), Confidor® (Imidacloprid) and Cymbush® (Cypermethrin) respectively. LC_{50} values for Politrin-C® and Talstar® 3.2 and 2.22 times lower than field recommended doses while Confidor® and Cymbush® 1.23 and 4.32 time higher than field recommended doses. These results indicated that Politrin-C® and Talstar® were more toxic after 24 hours intervals while Confidor® and Cymbush® were less toxic against citrus psylla, *Diaphorina citri* Kuwayama after 24 hours of exposure under laboratory conditions. Moghadam et al. (2011) tested that only last larval stage of Pistachio leaf white borer, *Ocneria terebinthina* was controlled by pyriproxyfen but other larval stages were not controlled by pyriproxyfen.

CONCLUSIONS

This study revealed that application of Politrin-C® (Profenofos + Cypermethrin) under laboratory and field condition proved to be more effective for the management of *Diaphorina citri*. Hence application of Politrin-C® was highly recommend for better management of *Diaphorina citri* and preferred for incorporation in IPM program.

AUTHORS' CONTRIBUTION

MI, SA and MDG designed the study, prepared layout, conducted experiments, BA and MJN collected the data, MI and MDB analyzed the data, all the authors helped in manuscript write up and formatting and MI and MDG proofread the manuscript.

CONFLICT OF INTEREST

The authors declare no conflict of interests.

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