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The efficacy of three plant leaves extract on Fall Army Worm, Cotton Mealy Bug and the Two Spotted Spider Mite

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Abstract
Fall army worm, *Spodoptera frugiperda* (FAW), (Lepidoptera: Noctuidae), is one of the most important invasive destructive pests in recent years which causes serious damages and a greet lose in crops. The cotton mealy bug, *Phenacoccus Solenopsis* Tinsley (Hemiptera: Pseudococcidae), and the two spotted spider mite, *Tetranychus urticae* Koch, (Acar: Tetranychidae), were both very important economic pest's world- wide. The aim of the study was to looking for environmentally friend, efficient and less cost plant leave extracts for controlling the above-mentioned pests. The plant leaves extract used in this study were, (Artichoke, Okra, Pepper) on the 2_{nd} and 4_{th} instar larvae of Fall army worm, *Spodoptera frugiperda* and the newly emerged adults of the cotton mealy bug, *Phenacoccus Solenopsis* Tinsley and two spotted spider mite, *Tetranychus urticae* Koch which, treated by four concentrations, (2000, 5000, 10000 & 20000 ppm) for each extract and the mortality was calculated after (1, 3, 5 and 7 days). The results showed that the higher concentration, (20000 ppm) of the Artichoke leaves extract found to had the highest total mortality percentage after 7 days of treatment for 2_{nd} and 4_{th} instar larvae of Fall army worm, *Spodoptera frugiperda*, (86.99 % & 38.67%), respectively. It also had the same effect on the two spotted spider mite, *Tetranychus urticae*, (98.00%), while for the cotton mealy bug, *Phenacoccus Solenopsis* Tinsley, it had the lowest effect, (55.33%). On the other hand, the highest total mortality percentage after 7 days for the cotton mealy bug, were when treated with Pepper leaves extract, (83.67%). In order to better understanding of the action of these extracts through studying their effect on biochemical parameters by estimating some enzymes activity.

Keywords: Plant leaves extract, *Spodoptera frugiperda*, *Phenacoccus Solenopsis* Tinsley, *Tetranychus urticae* Koch

Introduction

Spodoptera frugiperda, (Lepidoptera: Noctuidae), known as fall army worm (FAW), first invaded Africa in 2013 in Sao Tome and has since become established in many areas across the continent. It was found in Ghana and Sudan in 2017 and in Syria in 2020 and it can establish itself in almost all countries in central and eastern Africa and a large part of western Africa under this current climate. It was reported in Egypt in 2019 for the first time in maize field in Upper Egypt at a village in Kom Ombo in Aswan governorate. It infests more than 350 different plant species such as maize, broom corn, sugar cane, rice, cotton, vegetables and fruit. The importance of this pest came from its great destructive power and its short life cycle which not exceed a month and a half, so it can produce several generations per year and also the moth capable of flying over 100 Km / night. (Elnour *et al*., 2017, Goergen *et al*., 2016, Nada *et al*., 2012) [7, 12, 17] The cotton mealy bug *Phenacoccus solenopsis* Tinsley (Hemiptera: Pseudococcidae) considered one of the serious sap- sucking polyphagous pests causes major damage to many crops such as cotton (Elzahi *et al*., 2016, Mostafa *et al*., 2018) [9, 15], vegetables (Ibrahim *et al*., 2015) [14], many cultivated crops including weeds and many other field crops of economic importance which it feeds on all green parts of infested plants which made it weak and in sever infestation it leads to death (Culik and Gullan, 2005) [5].

The two spotted spider mite *Tetranychus urticae* Koch (Acari: Tetranychidae), is a world- wide polyphagous mite with the most economic importance due to its wide range of hosts and a high reproductive capacity and its ability to rabidly developing resistance to pesticides, and so it was found to be infesting a wide range of crops all over the world.

Due to the hazards and problems arising from application of pesticides for both human health and the Agro- system, the use of some safer means is been necessary. Nowadays plant extracts considered one of these much safer ways in controlling agricultural pests in many parts of the world as much easier and cheaper way for control. The current study was carried out to evaluate the efficacy of three plant leaves extract against, fall army worm, *Spodoptera frugiperda*, cotton mealy bug, *Phenacoccus solenopsis* Tinsley and the two spotted spider mite, *Tetranychus urticae* Koch.

Materials and methods

Rearing of tested insects and mite

Rearing of fall army worm, *Spodoptera frugiperda*

As a result of field observation on Okra plants at Barq- El Aize village, Mansoura district, Dakhlyia governorate, it found to be infested with fall army worm, *Spodoptera frugiperda*. Samples wear taken to laboratory for more inspections and larvae of different stages of (FAW), were collected and cultivated on maize leaves in laboratory till they reached pupal stage and then moths emergence and laying egg masses were then put in separated boxes. The newly hatched larvae were then fed on clean maize leaves at (25°C ± 2°C and 70% ± 5% Rh), till the 2_{nd} and 4_{th} instar and then each larval instar (2_{nd} and 4_{th}), were then treated with tested extracts. (Celis *et al*., 2014) [4].

Rearing of cotton mealy bug, *Phenacoccus solenopsis Tinsley*

The same Okra field at Barq- El Aize village, Mansoura district, Dakhlyia governorate, found also infested with the cotton mealy bug *Phenacoccus solenopsis* Tinsley, after observation it then transferred to the lap. The gravid females of the cotton mealy bug were inserted into sprouted potato was infested with only one adult female and observed daily. The newly hatched crawlers were then placed on each sprouted potato before being confined in a carton cylindrical box of 12 cm in diameter and 8 cm long, and the box was kept at (30℃ ± 2℃ and 60% ± 5% Rh). The box was examined daily for recording any morphological changed until the emergence of adults. The newly emerged adults then were treated with the tested extracts. (Attia & Ebrahim, 2015) [3].

Rearing of the two spotted spider mite, *Tetranychus urticae Koch*

The two spotted spider mite, *Tetranychus urticae* Koch, were collected from infested Okra plants at Barq- El Aize village, Mansoura district, Dakhlyia governorate. Samples were taken back to the lap then adult females were collected and placed on castor leaves upside down on cotton pads moisten with water in (12 cm diameter), petri- dishes and then kept in an incubator at (25℃ ± 2℃ and 70% ± 5% Rh). Daily the cotton pads were moistened to prevent the leaves from dryness and to prevent mites from escaping although leaves were replaced every 3 days till the emergence of the newly adult females which then were treated with the tested extracts. (Habashy *et al*., 2015) [13].

Plant leaves extracts: -

Selected plant leaves extracts were prepared at the laboratory, by Prof. Dr. Ghada E. Abd- Allah, plant protection research institute, Agriculture research center.

Toxicity bioassay

Dipping methods were used for all toxicity tests, where leaves were dipped in tested extracts and tap water as control for about 10 seconds, the treated leaves were left to dry at room temperature. Each plant leave extract (Artichoke, Okra & Pepper leave extracts), has four concentrations (2000, 5000, 10000 & 20000) ppm, each concentration had three replicates, where each replicate had (30, 2_{nd} or 4_{th} instar larvae of fall army worm), (30 newly emerged adults of cotton mealy bug) and (10 adult females of two spotted spider mite), counting live and dead individuals were done daily during inspections for 7 days. (Naglaa *et al*., 2023) [18].

Biochemical studies and Enzymes measurements

Samples were prepared by using the 2_{nd} instar larvae of fall army worm, newly emerged adults of cotton mealy bug and adult females of two spotted spider mite after 3 days of treated with LC50 level of Artichoke leaves extract and control. The samples were homogenized in distilled water and were centrifuged at 500 r. p. m. for 10 minutes at 5℃ to remove of the supernatants and hemocytes, then samples were analyzed to determine both alkaline and acid phosphatase activity. (Assar *et al*., 2012) [2].

Statistical analysis

The mortality percentages were calculated and corrected according to (Abbot, 1925) [1]. LC50 and LC90 were determined by statistical methods of Finney, 1971) [10]. LC50 index were determined by Sun equation (Sun, 1950) [20].

LC50 of the most effective compound

Toxicity index for LC_{50}= X 100

LC50 of the least effective compound

Results and discussion

I-The effect of three plant leaves extract on the 2_{nd} and 4_{th} instar larvae of fall army worm, *Spodoptera frugiperda*

Data obtained in table (1 &2) and fig. (1 &2) showed that Artichoke leaves extract at its higher concentration (20000 ppm) were more effective than Okra and Pepper leaves extract on both 2_{nd} and 4_{th} instar larvae which the total mortality percentage after 7 days of treatments were (8.99%, 71.33% & 54.67%), respectively for the 2_{nd} instar larvae, while these results decreased for the 4_{th} instar larvae but with the same trend which the total mortality percentage were, (38.67%, 21.34% & 15.99%), respectively.

Moreover, the LC50 for the three plant leaves extract for the 2_{nd} and 4_{th} instar larvae were, (5636.40 & 35755.82 – 4271.62 & 126437.24 – 18585.28 & 133116.43) ppm.

In addition to that the results also indicated that the toxicity index at LC50 was, (75.79%, 100% & 22.98), respectively for the 2_{nd} instar larvae, while, it was (100%, 28.28% & 26.886%) for the 4_{th} instar larvae respectively. These results agreed with (El- Sheikh, *et al*., 2021) [8], who studied the efficacy of some plant extracts on fall army worm in Sudan. It also agreed with (Mukanga, *et al*., 2022, Santos, 2012,) [16, 19].

Table 1: Corrected mortality % of 2 nd instar larvae of the fall armyworm *Spodoptera frugiperda*, treated with three different plant leaves extract

Treatments	Conc. (ppm)	Mortality after treatments %				Total Mortality %	LC_{50}	LC_{90}	P	R	Toxicity index
		1 day	3 days	5 days	7 days						
Artichoke leaves extract	2000	------	4.33	11.67	10.333	26.333	5636.40	31309.06	0.11	0.976	75.79
	5000	------	9.67	20.00	10.33	40.00					
	10000	4.67	12.00	24.33	22.67	63.67					
	20000	9.33	20.33	31.33	26.33	86.99					
Okra leaves extract	2000	------	10.67	12.00	20.33	43.00	4271.62	191047.05	0.38	0.958	100
	5000	------	14.33	17.67	14.33	46.33					
	10000	3.67	20.00	20.00	18.67	62.34					
	20000	6.67	24.33	20.00	20.333	71.33					
Pepper leaves extract	2000	------	------	6.67	10.00	16.67	18585.28	2428118.99	0.21	0.957	22.98
	5000	------	------	9.33	10.67	20.00					
	10000	3.33	10.67	12.33	10.33	36.66					
	20000	4.67	15.00	20.00	15.00	54.67					

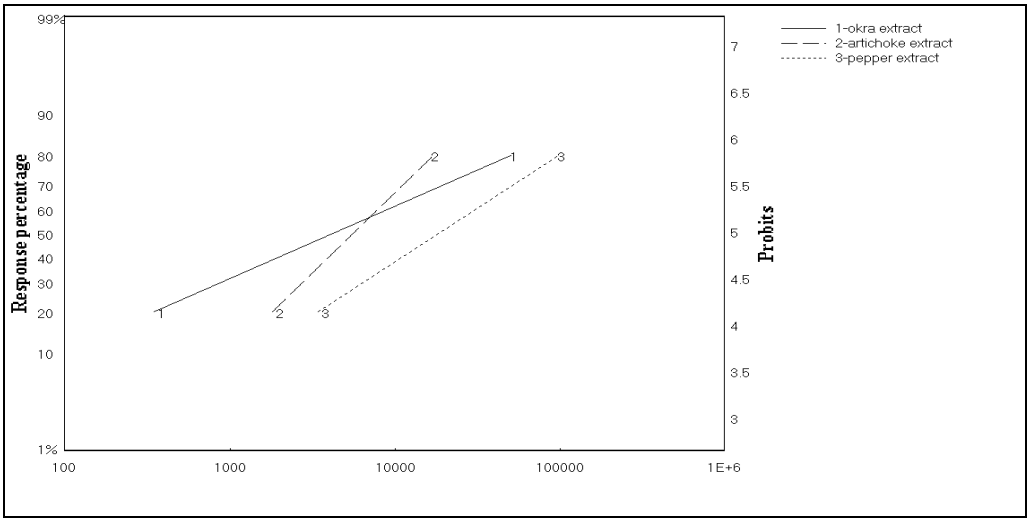

Fig 1: LD-P lines of the three plant leaves extract against 2nd instar larvae of the (FAW).

Table 2: Corrected mortality % of 4 th instar larvae of the fall armyworm *Spodoptera frugiperda*, treated with three different plant leaves extract

Treatments	Conc. (ppm)	Mortality after treatments %				Total Mortality %	LC_{50}	LC_{90}	P	R	Toxicity index
		1 day	3 days	5 days	7 days						
Artichoke leaves extract	2000	0.00	1.67	2.67	------	4.34	35755.82	250582.78	0.29	0.972	100
	5000	0.00	2.00	3.67	3.00	8.67					
	10000	3.33	6.33	3.67	2.67	16.00					
	20000	12.67	8.33	4.00	4.33	**38.67**					
Okra leaves extract	2000	0.00	2.33	1.00	------	3.33	126437.24	1916716.47	0.54	0.968	28.28
	5000	0.00	2.33	1.33	2.33	5.99					
	10000	3.33	3.67	2.00	------	9.00					
	20000	6.67	8.33	3.67	2.67	**21.34**					
Pepper leaves extract	2000	0.00	------	------	1.33	1.33	133116.43	1361632.26	0.74	0.986	26.86
	5000	0.00	------	2.67	1.00	3.67					
	10000	1.00	2.67	1.00	1.33	6.00					
	20000	4.33	3.33	4.33	4.00	**15.99**					

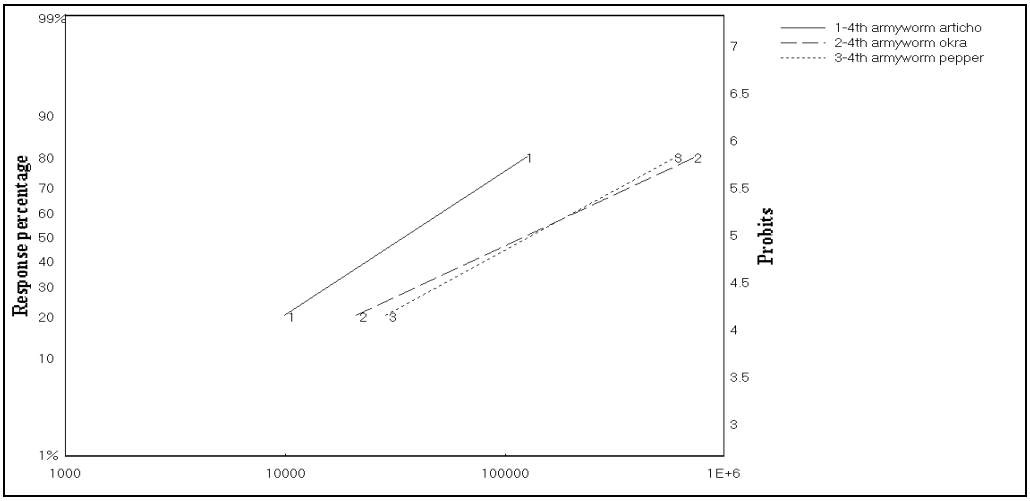

Fig 2: LD-P lines of the three plant leaves extract against 4th instar larvae of the (FAW).

II-The effect of three plant leaves extract on cotton mealy bug, *Phenacoccus solenopsis* Tinsley

Results in table (3) and fig. (3) illustrated that Pepper leaves extract with its highest concentration (20000 ppm) had the highest effect on *P. solenopsis* Tinsley, followed by Okra then Artichoke leaves extract which the total mortality percentages after 7 days of treatments was, (8.67%, 64.00% & 55.33%), respectively. In addition, the LC50 was, (1140.54, 7089.99 & 15404.69) ppm, respectively. while, the toxicity index at LC50 was, (100%, 16.09 & 7.40%) for Pepper, Okra and Artichoke leaves extract respectively.

These results agreed with (Ghada, 2022), [11] who studied the effect of globe Artichoke extract with and without adding Zamzam water on *P. solenopsis* Tinsley, also agreed with (Naglaa *et al*., 2023) [18], who indicated that Artichoke extract had the lowest effect on *P. solenopsis* Tinsley, from their other tested extracts.

Table 3: Corrected mortality % of the adults of cotton mealy bug *Phenacoccus solenopsis* Tinsley, treated with three different plant leaves extract

Treatments	Conc. (ppm)	Mortality after treatments %				Total Mortality %	LC_{50}	LC_{90}	P	R	Toxicity index
		1 day	3 days	5 days	7 days						
Artichoke leaves extract	2000	------	------	6.67	3.33	10.00	15404.69	130066.77			7.4
	5000	3.33	13.33	3.33	6.67	26.66					
	10000	13.33	10.00	10.00	6.67	40.00			0.86	0.997	
	20000	13.33	25.33	6.67	10.00	55.33					
Okra leaves extract	2000	7.67	4.33	10.00	10.33	32.33	7089.99	277726.64			16.09
	5000	9.00	15.33	16.00	6.00	46.33					
	10000	15.67	13.67	16.67	8.33	54.34			0.96	0.998	
	20000	21.00	15.67	12.00	15.33	64.00					
Pepper leaves extract	2000	13.33	26.67	11.00	6.33	57.33	1140.54	48641.35			100
	5000	13.33	33.33	20.00	3.33	69.99					
	10000	20.00	40.00	13.33	3.33	76.66			0.98	0.99	
	20000	20.00	40.33	16.67	6.67	**83.67**					

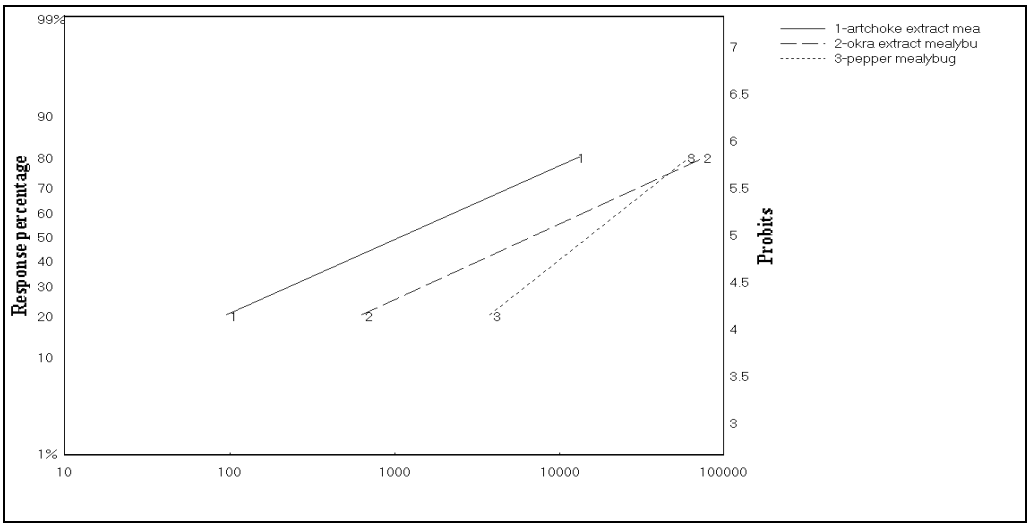

Fig 3: LD-P lines of the three plant leaves extract against Cotton mealybug

III-The effect of three plant leaves extract on the two spotted spider mite, *Tetranychus urticae* Koch

Data in table (4) and fig. (4) demonstrated that Artichoke leaves extract with the highest concentration, (20000 ppm) had the highest effect on *Tetranychus urticae* Koch where, the total mortality percentage after 7 days of treatment was, (98.00%) followed by Pepper leaves extracts, (80.67%) then the lowest total mortality percentage was for Okra leaves extract, (61.33%). Moreover, the LC50 was (2737.03 ppm, 3557.91 ppm and 9320.82 ppm), for Artichoke, Pepper and Okra leaves extract respectively.

On the other hand, the toxicity index was (100%, 29.37% and 76.93%), for Artichoke, Pepper and Okra leaves extract respectively. These results agreed with, (Yanar *et al*., 2011) [21], who studied the acaricidal effects of some plant parts extracts on *Tetranychus urticae* Koch.

Table 4: Corrected mortality % of the adult females of the two spotted spider mite *Tetranychus urticae* Koch, treated with three different plant leaves extract: -

Treatments	Conc. (ppm)	Mortality after treatments %				Total Mortality %	LC_{50}	LC_{90}	P	R	Toxicity index
		1 day	3 days	5 days	7 days						
Artichoke leaves extract	2000	4.67	25.33	7.33	3.33	40.66	2737.03	11637.73		0.986	100
	5000	7.33	27.67	25.33	9.00	69.33			0.39		
	10000	12.33	32.67	32.33	7.33	84.66					
	20000	34.67	18.00	35.33	10.00	98.00					
Okra leaves extract	2000	------	6.33	10.00	10.00	26.33	9320.82	221247.23			29.37
	5000	2.67	10.00	20.00	7.33	40.00					
	10000	3.67	20.00	17.33	11.33	52.33			0.96	0.989	
	20000	15.00	23.33	15.67	7.33	61.33					
Pepper leaves extract	2000	6.67	13.33	10.33	6.67	37.00	3557.91	42788.77			76.93
	5000	15.67	13.33	19.67	10.67	59.34					
	10000	6.33	26.67	22.33	14.67	70.00			0.84	0.996	
	20000	10.00	30.33	22.67	17.67	80.67					

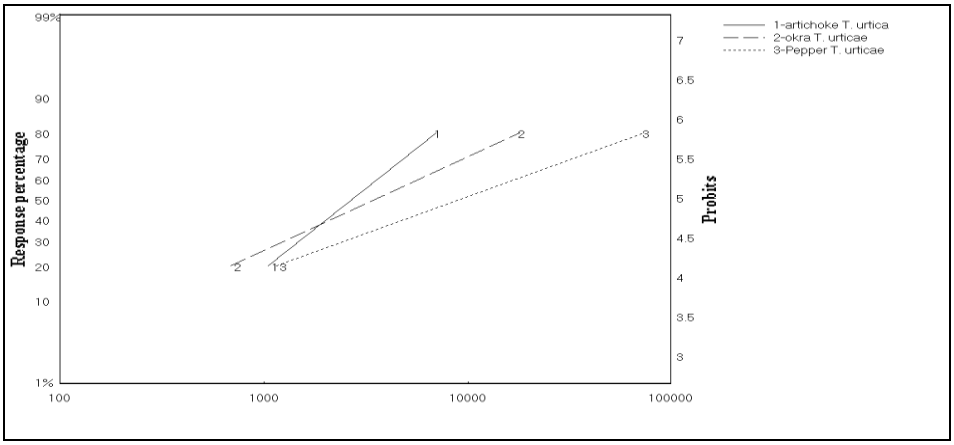

Fig 4: LD-P lines of the three plant leaves extract against Two spotted spider mite

Biochemical studies

From all the above-mentioned results that all indicated that Artichoke leaves extract had a high controlling effect for 2_{nd} instar larvae of (FAW) and the two spotted spider mite, while it had the opposite effect on the cotton mealy bug and for trying to understand why it had that effect the mean enzyme activity of both alkaline, (Alk- p) and acid phosphatase (AC- p) was measured as shown in table (5). The results illustrated that the Artichoke leaves extract causes an increase comparing with control in (Alk- p) activity which was, (26.52%, 20.00% and 182.71%) for 2_{nd} instar larvae of *S. frugiperda*, *P. Solenopsis* and *T. urticae*, respectively.

On the other hand, the Artichoke leaves extract caused decreased in (AC- p) activity which was, (-11.04% & -47.37%) for 2_{nd} instar larvae of *S. frugiperda* and *T. urticae*, respectively. While, it caused an increase in (AC- p) activity for *P. Solenopsis* which was, (7.65%) compared with control.

These results agreed with (El Barky *et al*., 2008) [6], but on *Spodoptera littoralis*.

Table 5: Alkaline and acid phosphatase activity in the hemolymph of 2 nd instar larvae *Spodoptera frugiperda*, adults of Phenacoccus *solenopsis* and Tetranychus *urticae*, after treatment with LC50 of artichoke leaves extract

	Mean enzyme activity (U/L/g.b. wt)	***Spodoptera frugiperda* 2 nd instar larvae**	***Phenacoccus solenopsis***	***Tetranychus urticae***
Alkaline phosphatase	Control	42.30	10.00	26.60
	After treatment	53.52	12.00	75.20
Changes %		26.52	20.00	182.71
Acid phosphatase	Control	10.33	1.70	1.90
	After treatment	9.19	1.83	1.00
Changes %		-11.***04***	7.65	-47.37

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