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ASIAN JOURNAL OF SCIENCE AND TECHNOLOGY

ISSN: 0976-3376

Asian Journal of Science and Technology
Vol. 12, Issue, 02, pp.11509-11513, February, 2021

RESEARCH ARTICLE

EFFECT OF PLASMA TREATMENT ON SEED GERMINATION AND GROWTH OF SELECTED CROP PLANTS

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ARTICLE INFO

Article History:
Received 07th November, 2020
Received in revised form
19th December, 2020
Accepted 24th January, 2021
Published online 28th February, 2021

Key words:

Vigna unguiculata L., Cajanus cajan L.Pisum sativum L.,
Cold plasma treatment.

ABSTRACT

Cold plasma treatment is widely used for activation and decontamination of surfaces. Owing to the unique plasma features this technique is applicable for modification of a wide range of thermally sensitive materials including biological tissues. Recently it has been applied successfully for treatment of plant seeds. In this paper releaved the comparison between untreated and plasma treated seeds of *Vigna unguiculata L. , Cajanus cajan L.Pisum sativum L.*in the different proportion of contaminated soil. In-vitro condition was used to find different morphological parameter of the plants. The plasma treated seeds shows excellent result than the untreated seeds.

Citation: ***Varna D. Trivedi, Falguni R. Patel and Nainesh R. Modi*. 2021.** "Effect of plasma treatment on seed germination and growth of selected crop plants", *Asian Journal of Science and Technology*, 12, (02), 11509-11513.

INTRODUCTION

Plasma is partially ionized gases also known as a highly-energized fourth state of matter that contains ions, electrons, and reactive neutral particles (radicals, and excited atoms and molecules), and sometimes with sufficient energy to break covalent bonds and/or initiate various chemical reactions (Sookwong *et al.,*2014). Magnetic field, ultraviolet, and other physical pathways are irradiating seeds as a traditional method to improve seed germination (Ling *et al.,* 2016). Based on that by reactors seeds can be treated by different kind of plasma for making seed more hydrophilic (Bormashenko *et al*., 2012). Application of cold plasma can be by two methods: direct treatment of seed and indirect treatment with plasma activated water of seed (Thirumdas *et al.,* 2018). Cold plasma treatment is widely used for activation and decontamination of surfaces. Owing to the unique plasma features this technique is applicable for modification of a wide range of thermally sensitive materials including biological tissues. (Filatova *et al.,* 2013). Recently, plasma has been applied to accelerate seed germination. For instance, air plasma treatment changes the wetting properties of seeds due to oxidation of their surface that leads to faster germination and greater yield. In recent times it has been applied successfully for treatment of plant seeds (Sookwong *et al.,* 2014).

*Pisum sativum L.*is commonly known as pea and it is a annual plant which growing upto 2 m in height. This leguminous food is a rich source of carbohydrate, protein, vitamins and minerals (Stola´rik *et al*., 2015 and Coasta *et al.,* 2006). The pea is an important vegetable crop due to its high nutritive value (Georgieva *et al.,* 2016). The seed is contraceptive, fungistatic and spermicidal. The dried and powdered of seeds has been used as a poultice on the skin where it has an appreciable effect on many types of skin complaint including acne. *Vigna unguiculata L.* is commonly called as Cowpea belongs to Leguminosae family (Ogbo *et al.,* 2009). Cowpea is now cultivated in all tropical areas and in some temperate areas such as the Mediterranean Basin, Iran, China and the southern states of the USA. (Pasquet *et al.,* 1998). Itis a annual climber and it has a capability to fix atmospheric nitrogen. Leaves of cowpea are applied to treat swellings and skin infections. Leaves are chewed to treat tooth ailments. The root is used as an antidote for snake bites and to treat epilepsy, chest pain, constipation and dysmenorrhea, and unspecified plant parts are used as a sedative in tachycardia and against various pains. *Cajanus cajan L.*is commonlyknown aspigeon pea and mostly cultivated in tropical and sub-tropical region of the world. The roots of pigeon pea having anthelmintic, sedative, expectorant and vulnerary properties. The leaves are used in treatment of pulmonary conditions such as coughs and bronchitis. The leaf juice is taken internally in the treatment of hemorrhages, coughs and diarrhea. The infusion of the leaves, combined with Dictyotene Aegyptus, is used to accelerate childbirth.

MATERIAL AND METHODOLOGY

Material

Plant material: Seeds of *Pisum sativum L , Cajanus cajan L. , Vigna unguculata L.* were purchased from certified seed selling shops only. Seeds of *Pisum stivum* was purchased from agro seeds, Randheja, Gandhinagar. *Cajanus cajan L.* seeds bought from Jaynit seeds private limited, Gandhinagar whereas, *Vigna uncuiculata L.* seeds got from Pethapur, Gandhinagar.

Methodology

Contaminated soil preparation

10g diesel contaminated soil

One kg soil has been taken+10-gram diesel (10-gram diesel =11.76 ml diesel) added.
↓
Same quantity of acetone added for even spreading of contaminant.
↓
Then soil was vigorously mixed for further use.

15g diesel contaminated soil

One kg soil has been taken+15-gram diesel (15-gram diesel =17.64m.l. diesel) added.
↓
Same quantity of acetone added for even spreading of contaminant.
↓
Then soil was vigorously mixed for further use.

35g diesel contaminated soil

One kg soil has been taken +35-gram diesel (35-gram diesel = 41.17 ml diesel) added.
↓
Same quantity of acetone added for even spreading of contaminant.
↓
Then soil was vigorously mixed for further use.

55g diesel contaminated soil

One kg soil has been taken +55-gram diesel (55- gram diesel = 64.70 ml diesel) added.
↓
Same quantity of acetone added for even spreading of contaminant.
↓
Then soil was vigorously mixed for further use.

Plasma treatment

Healthy looking seeds were selected.
↓
Seeds were put on still plate for treatment.
↓
Seeds were under goes grounded lower electrode for plasma treatment with selected treatment time.
↓
Seeds exposure to air plasma treatment for 10 min.
↓
Treated seeds taken for further sowing procedure.
↓
Plasma treated seeds (*Pisum sativumL., Cajanus cajan L. , Vigna unguculata L.)* socked in normal tap water for 2 hours.
↓
All seeds are socked in different contaminated soil *In vivo* condition.

RESULTS

The seeds of *Vigna unguiculata L. , Cajanus cajan L. Pisum sativum L.* are used in this experiment to find *In Vivo* condition different morphological parameters was studied which were mentioned below:-

1. Seed germination rate
2. Seedling's shoot length
3. Seedling's root length
4. Seedling's plantlet length
5. Plantlet's fresh weight
6. Plantlet's dry weight

Seed germination rate of *Vigna unguiculata L:* In *Vigna unguiculata L..,* the highest seed germination rate was observed in 10g contaminated soil (11.66%) as compare to, 15g (3.33%), 35g (6.66%), control (8.66%) and 55g (0%) in plasma treated seed after 5 weeks of completion (Table 3and Figure 4). While in plasma untreated seeds the highest seed germination rate was observed in control (8.66%) as compare to, 15g of contaminated soil (8%), 35g (8%) 10g (7.33%) and 55g (0%) after 5 weeks of completion (Table 1).

Seed germination rate of *Cajanus cajan L:* In *Cajanus cajan L.,* In untreated seeds the highest seed germination rate was observed in control (2.66%) as compare to , 15g of contaminated soil (2.66%) ,10g (1.66%), 35g (0.66%) and 55g (0%) after 5 weeks of completion while in plasma treated the highest seed germination rate was observed in 15g contaminated soil (3.33%) as compare to control (2.66%),10g (0.66%) , 35g (0.66%) and 55g (0%) in plasma treated seed after 5 weeks of completion (Table 2).

Seed germination rate of *Pisum sativum L:* In *Pisum sativum,* the highest seed germination rate was observed in control (17.33%) as compare to 10g contaminated soil, 15g (13.66%), 55g (10%) and 35g (13.66%) in untreated seed after 5 weeks of completion. While in plasma treated seeds the highest seed germination rate was observed in 10g of contaminated soil (18%) as compare to control (17.33%) , 15g (15.66%), 55g (13.33%) and 35g (12%) after 5 weeks of completion (Table 3).

Shoot length of *Vigna unguiculata L.* seeds: In untreated seeds of *Vigna unguiculata L.*, the highest shoot length was found in control (9.09cm) as compare to 10g contaminated soil (8.47cm), 15g (7.79cm), 35g (7.33 cm) and 55g (degraded after firat week) after 5 weeks of completion. In plasma treated seeds of *Vigna unguiculata L.*, the highest shoot length was observed in control (9.09cm) as compare to 10g of contaminated soil (7.06cm), 15g (4.75cm), 35g (4.66cm) and 55g (1cm) after 5 weeks of completion (Table 4)

Table 1. Germination rate of untreated and plasma treated seeds of *Vigna unguiculata L.*

Sr.no	Contaminated Soil	Untreated Germination rate of *Vigna unguiculata L.* (Percentage)					Plasma treated Germination rate of *Vigna unguiculata L.* (Percentage)				
		Week 1	Week 2	Week 3	Week 4	Week 5	Week 1	Week 2	Week 3	Week 4	Week 5
1	Control	4.66	7.33	8.66	8.66	8.66	4.66	7.33	8.66	8.66	8.66
2	10 g diesel	4.66	6.33	6.66	6.66	7.33	7.33	10	11.33	11.66	11.66
3	15 g diesel	4.66	7.33	8	8	8	2.33	2.33	2.66	3.33	3.33
4	35 g diesel	5.33	7.33	8	8	8	3.33	3.66	6	6.66	6.66
5	55 g diesel	0.33	0.66	1.33	-	-	0.33	0.66	0.66	0.66	0

Table 2. Germination rate of untreated and plasma treated seeds of *Cajanus cajan L.*

Sr.no	Contaminated Soil	Untreated Germination rate of *Cajanus cajan L.*(Percentage)					Plasma treated Germination rate of *Cajanus cajan L.*(Percentage)				
		Week 1	Week 2	Week 3	Week 4	Week 5	Week 1	Week 2	Week 3	Week 4	Week 5
1	Control	0	2.66	2.66	2.66	2.66	0	2.66	2.66	2.66	2.66
2	10 g diesel	0	1.33	1.66	1.66	1.66	0.66	0.66	0.66	0.66	0.66
3	15 g diesel	0	0	0.66	2	2.66	2	4	3.33	3.33	3.33
4	35 g diesel	0.66	0.66	0.66	0.66	0.66	0.66	0.66	0.66	0.66	0.66
5	55 g diesel	-	-	-	-	-	-	-	-	-	-

Table 3. Germination rate of untreated and plasma treated seeds of *Pisum sativum L.*

Sr.no	Contaminated Soil	Untreatedgermination rate of*Pisum sativum L.*(Percentage)					Plasma treated germination rate of *Pisum sativum L.* (Percentage)				
		Week 1	Week 2	Week 3	Week 4	Week 5	Week 1	Week 2	Week 3	Week 4	Week 5
1	Control	12.66	13.44	14.66	16	17.33	12.66	18	14.66	16	17.33
2	10 g diesel	8	13.33	15.33	15.66	16	9.33	11.33	16	16	18
3	15 g diesel	7.33	10	13.33	13.66	13.66	10	14.66	13.33	15.33	15.66
4	35 g diesel	10	12	12.66	13.33	13.66	7.33	8	10	12	12
5	55 g diesel	4	7.3	8	10	10	6	6.66	9.33	9.33	13.33

Table 4. Shoot length of untreated and plasma treated seeds of *Vigna unguiculata L.*

Sr. no	Contamin-ated Soil	UntreatedShoot length of *Vigna unguiculata L.* (Percentage)					Plasma treatedShoot length of *Vigna unguiculata L.* (Percentage)				
		Week 1	Week 2	Week 3	Week 4	Week 5	Week 1	Week 2	Week 3	Week 4	Week 5
1	Control	1 ± 0.31	3.96 ± 1.71	4.65 ± 2.60	7.90 ± 3.38	9.09 ± 3.36	1 ± 0.31	3.96 ± 1.71	4.65 ± 2.60	7.90 ± 3.38	9.09 ± 3.36
2	10 g diesel	0.78 ± 0.26	3.77 ± 1.66	5.9 ± 1.66	6.9 ± 1.79	8.47 ± 1.81	0.55 ± 0.35	2.63 ± 1.43	2.62 ± 2.09	5.12 ± 2.98	7.06 ± 3.06
3	15 g diesel	0.64 ± 0.45	2.72 ± 1.44	3.79 ± 0.94	6.5 ± 2.81	7.97 ± 3.15	0.5 ± 0.43	2.63 ± 1.43	2.75 ± 2.36	3.33 ± 2.51	4.75 ± 2.36
4	35 g diesel	0.26 ± 0.074	1.92 ± 1.55	3.02 ± 2.18	6 ± 2.81	7.33 ±2.91	0.1 ± 0.08	1.22 ± 1.08	2.22 ± 1.67	4 ±1.41	4.66 ± 2.51
5	55 g diesel	0.28 ± 0.29	-	-	-	-	0.2 ± 0.14	1	1.5	3	1

Table 5. Shoot length of untreated and plasma treated seeds of *Cajanus cajan L.*

Sr. no	Contaminated Soil	Untreated Shoot length of *Cajanus cajan L.*(Percentage)					Plasma treated Shoot length of *Cajanus cajan L.* (Percentage)				
		Week 1	Week 2	Week 3	Week 4	Week 5	Week 1	Week 2	Week 3	Week 4	Week 5
1	Control	0.7 ± 0.42	1.5 ± 1.68	2.33 ± 1.04	8.16 ± 4.01	10 ± 4.02	0.7 ± 0.42	1.5 ± 1.68	2.33 ± 1.04	8.16 ± 4.01	10 ± 4.01
2	10 g diesel	1	0.75 ± 0.35	1	2	4	-	1	1	1.5	2
3	15 g diesel	0.46 ± 0.46	1	1	1.66 ± 0.57	2.5 ± 0.5	-	1.33 ± 0.75	3.1 ± 1.88	4.1 ± 1.67	5.66 ± 1.86
4	35 g diesel	1	1	1	-	-	0.2	-	-	-	-
5	55 g diesel	-	-	-	-	-	-	-	-	-	-

Shoot length of *Cajanus cajan L* seeds: In plasma treated seeds of *Cajanus cajan L.*, the highest shoot length was observed in control (10cm) as compare to 15g of contaminated soil (11.33cm), 10g (10.66cm). In 35g and 55g of contaminated soil there was no results after 5 weeks of completion. (Table 11 and figure 12).While in untreated seeds of *Cajanus cajan L.*, the highest shoot length was found in control (10cm) as compare to 10g contaminated soil (4cm), 15g (2.5cm), 35g and 55gof contaminated soil didn't showed results after 5 weeks of completion. (Table 5)

Shoot length of *Pisum sativum L*. seeds: In plasma treated seeds of *Pisum sativum L.*, the highest shoot length was observed in control (13.8cm) as compare to 15g of contaminated soil (11.33cm), 10g (10.66cm), 35g (7.66cm) and 55g (5.63cm) after 5 weeks of completion. While in untreated seeds of *Pisum sativum L.*, the highest shoot length was found in control (13.87cm) as compare to 10g contaminated soil (13.77cm), 15g (12.75cm), 35g (11.34 cm) and 55g (10.46cm) after 5 weeks of completion. (Table 6)

Root length of *Vigna unguiculata L., Cajanus cajan L.Pisum sativum L.* seeds: The highest root length was observed in seeds of *Pisum sativum L.*in bothplasma treated and untreated (12.18cm) in control condition and in plasma treated seeds of *Vigna unguiculata L.*showed highest root length in 10g contaminated soil (7.13cm) where as in untreated seeds of *Cajanus cajan L.*indicated highest root length (7cm) in 15g contaminated soil (Table 7).

Plantlet length of *Vigna unguiculata L., Cajanus cajan L.Pisum sativum L.*seeds: The highest plant length was observed in seeds of *Pisum sativum L.* in bothplasma treated and untreated (27.21cm) in control condition and in plasma treated seeds of *Vigna unguiculata L.* showed highest plant length in 35g contaminated soil (18.33cm) where as in untreated seeds of *Cajanus cajan L.*indicated highest plant length (17.16cm) in 10g contaminated soil. (Table 8)

Table 6. Shoot length of untreated and plasma treated seeds of *Pisum sativum L.*

Sr. no	Contaminated Soil	Untreated Shoot length of *Pisum sativum L.* (Percentage)					Plasma treated Shoot length of *Pisum sativum L* (Percentage)				
		Week 1	Week 2	Week 3	Week 4	Week 5	Week 1	Week 2	Week 3	Week 4	Week 5
1	Control	1.25 ± 0.76	5.44 ± 2.79	8.70 ± 2.05	11.81 ± 3.53	13.87 ± 4.041	1.25 ± 0.76	5.44 ± 2.79	8.70 ± 2.05	11.81 ± 3.53	13.8 ± 0.41
2	10 g diesel	1.18 ± 0.55	3.35 ± 1.98	8.36 ± 3.43	12.72 ± 3.10	13.77 ± 3.35	0.66 ± 0.45	5.3 ± 2.85	8.36 ± 3.43	9.16 ± 4.55	10.66 ± 3.78
3	15 g diesel	1.36 ± 0.86	4.03 ± 3.24	7.1 ± 3.94	11.43 ± 4.30	12.75 ± 4.73	0.88 ± 0.40	4.21 ± 1.72	7.13 ±2.39	7.42 ± 4.50	11.33 ± 2.88
4	35 g diesel	1.26 ± 0.45	4.73 ± 1.32	7.13 ± 2.3	9.84 ± 3.63	11.34 ± 3.82	0.99 ± 0.86	2.91 ± 2.44	7.13 ± 2.39	7.42 ± 4.50	7.66 ± 2.88
5	55 g diesel	0.61 ± 0.42	3.09 ± 1.30	4.87 ± 2.74	8.9 ± 4.58	10.46 ± 3.58	1.12 ± 0.87	2.65 ± 1.88	4.87 ± 2.74	5.17 ± 1.83	5.63 ± 3.78

Table 7. Root length of untreated and plasma treated seeds of *Vigna unguiculata L., Cajanus cajan L.Pisum sativum L.*

Sr. no	Contaminated soil	Root length of *Vigna unguiculata L.*(cm)		Root length of*Cajanus cajan L.*(cm)		Root length of *Pisum sativum L.*(cm)	
		Untreated	Plasma treated	Untreated	Plasma treated	Untreated	Plasma treated
1	Control	6.86 ± 2.80	6.86 ± 2.80	-	-	12.18 ± 5.56	12.18 ± 5.56
2	10 g diesel	3.75 ± 1.35	7.13 ± 2.91	-	-	10.42 ± 4.55	10.73 ± 4.80
3	15 g diesel	5 ± 2.34	3.33 ± 0.57	1.41 ± 1	7 ± 2.64	11.21 ± 5.05	9.93 ± 5.26
4	35 g diesel	6.8 ± 3.03	4	-	-	7.65 ± 3.39	8.36 ± 4.07
5	55 g diesel	-	-	-	-	8.77 ± 4.06	5.53 ± 3.19

Table 8. Plantlet length of untreated and plasma treated seeds of *Vigna unguiculata L., Cajanus cajan L. Pisum sativum L.*

Sr. no	Contaminated Soil	Plantlet length of *Vigna unguiculata L.*(cm)		Plantlet length of*Cajanus cajan L.*(cm)		Plantlet length of *Pisum sativum L.* (cm)	
		Untreated	Plasma treated	Untreated	Plasma treated	Untreated	Plasma treated
1	Control	14.96 ± 6.01	14.96 ± 6.02	-	-	27.21 ± 8.05	27.21 ± 8.05
2	10 g diesel	17.54 ± 5.39	12.16 ± 2.63	-	17.16 ± 7.18	23.95 ± 9.03	23.95 ± 9.03
3	15 g diesel	10.33 ± 1.52	12.64 ± 5.91	9.33 ± 3.78	10	22.03 ± 8.50	22.03 ± 8.50
4	35 g diesel	11	18.33 ± 6.50	-	-	20.80 ± 5.65	20.80 ± 5.65
5	55 g diesel	-	-	-	-	21.22 ± 8.83	21.22 ± 8.83

Table 9. Fresh weight of untreated and plasma treated seeds of *Vigna unguiculata L., Cajanus cajan L. Pisum sativum L.*

Sr. no	Contaminated Soil	Fresh weight of *Vigna unguiculata L.*(cm)		Fresh weight of*Cajanus cajan L.*(cm)		Fresh weight of *Pisum sativum L.* (cm)	
		Untreated	Plasma treated	Untreated	Plasma treated	Untreated	Plasma treated
1	Control	2.6 ± 0.52	2.6 ± 0.52	2.43 ± 0.81	2.43 ± 0.81	5.66 ± 2.51	5.66 ± 2.51
2	10 g diesel	2.08 ± 0.84	1.1 ± 0.60	1.2	-	7.66 ± 3.78	5.33 ± 2.30
3	15 g diesel	5.3 ± 5.89	1.53 ± 0.64	1.2 ± 0.42	2.41 ± 0.42	9.33 ± 8.38	4.66 ± 1.52
4	35 g diesel	1.07 ± 0.37	1.4 ± 0.95	-	-	3.66 ± 2.88	3
5	55 g diesel	0.73 ± 0.68	0.23 ± 0.14	-	-	2.63 ± 3.78	1.30 ± 0.60

Table 10: Dry weight of untreated and plasma treated seeds of *Vigna unguiculata L. , Cajanus cajan L.Pisum sativum L.*

Sr. no	Contaminated Soil	Wet weight of *Vigna unguiculata L.*(cm)		Wet weight of *Cajanus cajan L.*(cm)		Wet weight of *Pisum sativum L.*(cm)	
		Untreated	Plasma treated	Untreated	Plasma treated	Untreated	Plasma treated
1	Control	0.38 ± 0.50	0.38 ± 0.50	0.50 ± 0.07	0.50 ± 0.07	2.41 ± 1.71	2.41 ± 1.71
2	10 g diesel	-	0.55	0.66 ± 0.27	0.37 ± 0.23	1.80 ± 0.98	1.69 ± 0.90
3	15 g diesel	0.02 ± 0.01	0.55 ± 0.42	1.82 ± 1.60	0.17 ± 0.06	5.66 ± 2.69	1.52 ± 0.37
4	35 g diesel	-	-	0.23 ± 0.31	0.39 ± 0.37	1.23 ± 0.73	1.03 ± 0.28
5	55 g diesel	-	-	0.08 ± 0.10	0.05 ± 0.03	0.61 ± 0.42	0.57 0.30

Fresh weight of *Vigna unguiculata L., Cajanus cajan L. Pisum sativum L.* seeds: The maximum fresh weight was found in untreated seeds of *Pisum sativum L.*(9.33g) in 15g contaminated soil than in untreated seeds of *Vigna unguiculata L.*showed maximum fresh weight (5.3g) in 15g contaminated soil where as in control condition the seeds of *Cajanus cajan L.*indicated maximum fresh weight (2.43g) in both plasma treated and untreated (Table 9).

Dry weight of *Vigna unguiculata L., Cajanus cajan L.Pisum sativum L.* seeds: The maximum dry weight was found in untreated seeds of *Pisum sativum L.*(5.66g) in 15g contaminated soil than in untreated seeds of *Vigna unguiculata L.* showed maximum dry weight (1.82g) in 15g contaminated soil where as in control condition the seeds of *Cajanus cajan L.* indicated maximum dry weight (0.55g) 15 g in untreated and (0.38) control plasma treated (Table 10).

CONCLUSION

In the contaminated soil, contaminated diesel fuel with different concentrations (10 gm., 15 gm., 35 gm., and 55gm.) i.e. are added in one kilograms soil shows effect on plants growth parameters such as seed germination rate , shoot length , root length , fresh weight and dry weight. Plasma treatment is also significant here. In *Pisum sativum L.,* shoot length is significant in plasma treated plants while in plasma untreated it's lesser than plasma treated plants. In *Vigna unguiculata L.,* the shoot length is increased in treated plants where in plasma untreated one were negleable difference. But in control (plasma untreated seeds) showing high rate of germination and uniform germination in all contamination of soil. In the *Cajanus cajan L.,* it did not showed well germination neither in treated nor in untreated one , but untreated plants showing well growth in shoot compared to treated plants. Plasma untreated (control) shows good results in this species. Both plasma treated and plasma untreated (without any treatment) seeds of taken all species showed germination in all concentrations of diesel contaminated soil which suggest that diesel contamination can only delay seed germination but also affect other morphological parameters. By time with temperature diesel can evaporate in air and clear the way of germination by providing essentials to seed. And that can be said why seeds got germinated in contaminated soil.

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