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RESEARCH ARTICLE

EVALUATION OF ALLELOPATHIC POTENTIAL OF *DELONIX REGIA* AGAINST GERMINATION PHYSIOLOGY OF MUNG BEAN SEEDLING

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ARTICLE INFO ABSTRACT In India, mungbean is the 3rd major pulse crop cultivated for best source of proteins, carbohydrates Article History: and minerals. Seed germination and germination physiology play an important role in crop production. Received 18th March, 2017 Ornamental plant Delonix regia is planted as shade tree in farm competes with neighboring plant Received in revised form species. Phytotoxins released by dry, dropped leaves of Delonix may have adverse effects on 03rd April, 2017 Accepted 24th May, 2017 neighboring crop plants. Hence a study was carried out to evaluate the effect of aqueous leachates of Published online 30th June, 2017 leaves of D. regia on germination and some metabolic facets of seedlings of mungbean. In petriplate bioassay root length (74.62%, 17.20%, and 47.31%) and shoot length (76.43%, 16.98% and 46.70%) Key words: were reduced due to 20%, 0.2%, and 0.1% respectively. In soil bioassay, decrease in root length (16.09%, 13.73%, 1.02%) was observed. In petriplate bioassay a decrease in starch content (14.55%, Delonix regia, 10.97%, 36.42%), reducing sugar (60.94%, 67.45%, 17.45%), total sugar (43.50%, 57.62%, 18.25%) Leaf leachate, and increase in protein content (54.26%, 13.42%, 42.61%) was observed due to treatment of leaf Phytochemical analysis, leachates. In soil bioassay, there was increase in starch (42.24%, 1071.12%, 859.66%) and total sugar Seedling growth, Vigna radiata. content (20.06%, 63.78%, 23.81%) and decrease in reducing sugar content (32.93%, 3.44%, 15.17%) and soluble protein content (3.64%, 0.298%) in seedlings of mung bean. Leaf leachate showed

seedling growth and alteration in metabolism of mungbean seedlings may be due to the synergistic effect of appreciable amount of polyphenols and phytochemicals present in the leaf leachates.

considerable amount of polyphenols and number of phytochemicals. Inhibition of seed germination,

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INTRODUCTION

Seed germination plays a significant role in the life cycle of crop plants, as well as for successful crop production. It sustains crop reproduction and restrains the dynamic of crop plants population (Dash, 2012). In germination of crop plants, seedling growth and physiological processes may be positively or negatively altered by the factors which are associated with climatic changes (Reddy et al., 2000). Mijani et al. (2013) reported that successful establishment of plant species is dependent on adaptive mechanisms of seed germination and seedling growth to hostile climatic conditions. Germination, growth and yield of crop plants may be distressed by the phytotoxins released by higher plants (Singh et al., 2006). Allelopathic agents affect the performance of plant by impeding seed germination, root length or plant growth (Djlirdjevic et al., 2007). In plants, allelochemicals affect the physiological processes like cell division, elongation,

membrane permeability, plant nutrient uptake, photosynthesis, respiration, enzyme activities and protein synthesis (John and Sarada, 2012). In agroforestry programme, allelopathic interactions in tree-crop association crop production may be affected. A variety of secondary metabolites found in crop field, which are liberated by the tree species, may hamper germination and growth of crops (Singh et al., 2006). In India, mung bean is the third major pulse crop grown on 3.72 million ha area having about 1.56 million ton production (Abdel Haleem, 2007). Mung bean which is cultivated for its dry edible seeds and fresh sprouts is considered one of the best source of proteins, carbohydrates and minerals (Sehrawat et al., 2013). Though Delonix regia is planted as ornamental plant and shade tree in farms, it competes with neighboring plant species and turns land under its canopy barren (Orwa et al., 2009). Shedding of dried leaves of Delonix is observed frequently. These dried leaves may get accumulated in farms and may have antagonistic effect on crop plant species due to release of phytochemicals. In the light of the above the present study was carried out to evaluate allelopathic potential of D. regia against germination physiology of Vigna radiata.

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Germination percentage						
24hrs		48hrs		72hrs		
	Percent increase		Percent increase		Percent increase	
	(+)/decrease (-)		(+)/decrease (-)		(+)/decrease (-)	
75±15.16***	-	77±16.53***	-	80±15.49***	-	
20±12.65**	73.33 (-)	48±14.14***	37.66 (-)	48±11.69***	40 (-)	
42±23.16***	44 (-)	65±10.49***	15.58 (-)	67±12.11***	16.25(-)	
48±27.14***	36 (-)	57±23.38***	25.97 (-)	70±10.95***	12.5 (-)	
	24hrs 75±15.16*** 20±12.65** 42±23.16***	24hrs Percent increase (+)/decrease (-) 75±15.16*** 20±12.65** 73.33 (-) 42±23.16*** 44 (-)	24hrs 48hrs Percent increase (+)/decrease (-) 77±16.53*** 75±15.16*** - 77±16.53*** 20±12.65** 73.33 (-) 48±14.14*** 42±23.16*** 44 (-) 65±10.49***	24hrs 48hrs Percent increase (+)/decrease (-) Percent increase (+)/decrease (-) 75±15.16*** - 20±12.65** 73.33 (-) 48±14.14*** 37.66 (-) 42±23.16*** 44 (-)	$\begin{array}{c ccccccccccccccccccccccccccccccccccc$	

Table 1. Effect of leaf leachate of D. regia on seed germination of Vigna radiata (Petriplate bioassay)

Significantly different at * 1% level, ** 5% level, *** 1% and 5% level, NS- Non significant

Table 2. Effect of leaf leachate of *D. regia* on seedling growth of *Vigna radiata* (Petriplate bioassay)

Treatment	Seedling growth			
	Average root length (cm)	Average shoot length (cm)
]	Percent increase (+)/ decrease (-)		Percent increase (+)/ decrease (-)
Control	4.65±0.65***	-	4.71± 0.64***	-
20% leaf leachate	1.18± 0.79**	74.62 (-)	1.11±0.72**	76.43 (-)
0.2% leaf leachate	$3.85 \pm 0.70 ***$	17.20 (-)	$3.91 \pm 0.69 ***$	16.98 (-)
0.1% leaf leachate	2.45±0.71***	47.31 (-)	2.51±0.64***	46.70 (-)

Significantly different at * 1% level, ** 5% level, *** 1% and 5% level, NS- Non significant

Table 3. Effect of leaf leachate of *D. regia* on seedling growth of *Vigna radiata* (Soil bioassay)

Treatment	Seedling growth			
	Average root length ((cm)	Average shoot le	ength (cm)
		Percent increase (+)/decrease (-)		Percent increase (+)/decrease (-)
Control	13.61±2.81***	-	13.39±1.59***	-
20% leaf leachate	11.42±2.42***	16.09 (-)	12.09±2.49***	9.70 (-)
0.2% leaf leachate	11.74±2.11***	13.73 (-)	12.42±1.47***	7.24 (-)
0.1% leaf leachate	13.47±4.99**	1.02 (-)	14.04±2.40***	4.85 (+)

Significantly different at * 1% level, ** 5% level, *** 1% and 5% level, NS- Non significant

Table 4. Effect of leaf leachate of D. regia on starch content in seedling of Vigna radiate

Treatment	Starch content (mg	g ⁻¹ d. wt.)		
	Petriplate bioassay		Soil bioassay	
		Percent increase (+)/decrease (-)		Percent increase (+)/decrease (-)
Control	12.85±1.76***	-	8.38±1.59**	-
20% leaf leachate	10.98±1.21***	14.55 (-)	11.92±1.79***	42.24 (+)
0.2% leaf leachate	11.44±1.48***	10.97 (-)	98.14± 9.64***	1071.12 (+)
0.1% leaf leachate	8.17±0.90***	36.42 (-)	80.42±1.38***	859.66 (+)

Significantly different at * 1% level, ** 5% level, *** 1% and 5% level, NS- Non significant

Table 5. Effect of leaf leachate of D. regia on reducing sugar content in seedling of Vigna radiata

Treatment	Reducing sugar content (mg g ⁻¹ d. wt.)				
	Petriplate bioassay				
		Percent increase (+)/decrease (-)		Percent increase (+)/decrease (-)	
Control	0.676±0.067***	-	1.16±0.068***	-	
20% leaf leachate	0.264±0.044***	60.94 (-)	0.778±0.051***	32.93 (-)	
0.2% leaf leachate	0.22±0.13NS	67.45 (-)	1.12±0.16***	3.44 (-)	
0.1% leaf leachate	0.558±0.15**	17.45 (-)	0.984±0.067***	15.17 (-)	

Significantly different at * 1% level, ** 5% level, *** 1% and 5% level, NS- Non significant

Table 6: Effect of leaf leachate of D. regia on total sugar content in seedling of Vigna radiata

Treatment	Total sugar conten	Total sugar content (mg g ⁻¹ d. wt.)			
	Petriplate bioassay	Petriplate bioassay Sc			
		Percent increase (+)/decrease (-)		Percent increase (+)/decrease (-)	
Control	0.8±0.1***	-	1.331±0.085***	-	
20% leaf leachate	0.452±0.037***	43.50 (-)	1.598±0.087***	20.06 (+)	
0.2% leaf leachate	0.339±0.10**	57.62 (-)	2.18±0.12***	63.78 (+)	
0.1% leaf leachate	0.654±0.18**	18.25 (-)	1.648±0.12***	23.81 (+)	

Significantly different at * 1% level, ** 5% level, *** 1% and 5% level, NS- Non significant

Soluble protein content (mg g ⁻¹ d. wt.)			
Petriplate bioassay Soil bi		Soil bioassay	
	Percent increase (+)/decrease (-)		Percent increase (+)/decrease (-)
70.45±2.45***	-	87.22±2.79***	-
108.68±3.30***	54.26 (+)	84.04±1.42***	3.64 (-)
79.91±1.30***	13.42 (+)	86.96±0.84***	0.298 (-)
100.47±1.42***	42.61 (+)	90.06±5.08***	3.25 (+)
	Petriplate bioassay 70.45±2.45*** 108.68±3.30*** 79.91±1.30***	Petriplate bioassay Percent increase (+)/decrease (-) 70.45±2.45*** 108.68±3.30*** 54.26 (+) 79.91±1.30*** 13.42 (+)	Petriplate bioassay Soil bioassay Percent increase (+)/decrease (-) 70.45±2.45*** - 87.22±2.79*** 108.68±3.30*** 54.26 (+) 84.04±1.42*** 79.91±1.30*** 13.42 (+) 86.96±0.84***

Table 7. Effect of leaf leachate of D. regia on soluble protein content in seedling of Vigna radiata

Significantly different at * 1% level, ** 5% level, *** 1% and 5% level, NS- Non significant

Table 8. Chemical analysis of leaf leachate

Nitrate content (mg g^{-1} d. wt.)	Nitrite content (mg g ⁻¹ d. wt.)	Total polyphenol (mg g ⁻¹ d. wt.)	Phytochemicals detected
22.67±1.41***	0.048±0.003***	4.547±0.367***	Camphene; 2-Methyl-2-hepten-6-one; (1S)-2,6,6-Trimethylbicyclo(3.1.1) hept-2-ene; 1R-alphapinene; Ocimene; Dimethyloctatriene; Alpha-Fenchene; 4-Nonanone; 7- Methyl-4-octanone; 2-Methyl-3-octnone; 2, 6- Dimethyl-3-heptanone, Linalol, 2,4,6-Trimethyl-3- Cyclohexen-1-Carboxyaldehyde; Artemiseole; 2-Isopropenyl-5 methylhex-4-enal; (E)-3(10)-Caren-2-ol; Cis- Geraniol; Alpha-citral; Beta-citral; Neryl acetate; Caryophyllene; Germacrene; Copaene

Significantly different at * 1% level, ** 5% level, *** 1% and 5% level, NS- Non significant

MATERIALS AND METHODS

Procurement of plant material

In the month of November and December, 2013, dried and dropped leaves were hoarded from streets and farm sides in Kolhapur District (Maharashtra State, India). Certified seeds of mung bean (*V. radiata* (L.) R. Wilczek variety SML-668) (Ajinkya Seeds, Pune) were purchased from local market.

Formulation and chemical analysis of leaf leachate

Consistent with the method of Jadhav and Gaynar (1992), 10 grams of leaves sluiced 4-5 times with distilled water and soaked in 50ml distilled water for 24 hrs. Filtration of leaf leachate followed by dilution of 20% leachate to 0.1% and 0.2% concentrations. For assessment of nitrate content, 200µl 20% leachate was treated with salicylic acid-sulphuric acid and then with 2N NaOH to raise the pH above 12 and absorbance was recorded at 420nm (Cataldo et al. 1975). For nitrite determination leachate was treated with 1% Sulfanilamide and 0.02% NEEDA and absorbance was recorded at 540nm (Nair et al. 1988). As per the method of Folin and Denis (1915) total polyphenol content in leaf leachate was estimated by reaction between leachate and Folin Denis reagent and 20% Na₂CO₃ and recording absorbance at 660nm. Nitrate, nitrite and polyphenol content were expressed as mg per g dry weight with the help of standard curve. GC-MS technique utilized to detect phytochemicals in methanolic leachate of leaves.

Seed germination and growth study

In petriplate bioassay, for surface sterilization healthy mungbean seeds treated with 0.1% HgCl₂ and rinsed with distilled water. Each sterile petriplate having 10 seeds supplied with 8ml of leachates (20%, 0.1% and 0.2%) and 8ml distilled water for treatment and control respectively. Seeds were allowed to germinate at room temperature (24-28°C) under light and dark cycles. To note germination seeds at 24, 48 and 72hrs stage and for seedling growth 120hrs stage was considered. For soil bioassay, plastic trays (dimension 22cm x 17cm x 4.2cm) filled with 750 grams of dry soil.

Thirty seeds were sown in moistened soil. Fifty ml of leachate and 50 ml of distilled water applied for 5 days (at alternate day). Root length and shoot length of 10 days old seedlings was noted.

Estimation of starch, sugars and protein

For determination of starch and sugar content ethanolic extract of dry seedlings and residue was used. For reducing sugar content filtrate was condensed and decolorized (Lead acetate + Potassium oxalate, 1:1 proportion) and filtered after addition of distilled water. For total sugar and starch, 20ml of filtrate and residue hydrolyzed by autoclaving with concentrated HCl. Contents were neutralized with Na₂CO₃ and filtered. For evaluation of starch and sugar contents filtrates were treated with Somogyi's alkaline copper tartarate reagent(4 g CuSO₄. 5H₂O, 24 g anhydrous Na₂CO₃, 16 g Na-K-tartarate and 180 g anhydrous Na₂SO₄ dissolved in 1 liter distilled water) and Arsenomolybdate reagent (25 g Ammonium molybdate dissolved in 450 ml distilled water, 3 g sodium arsenate dissolved in 25 ml distilled water, 21 ml concentrated HCl. These ingredients were mixed well and digested for 48 hours at 37°C) and values were expressed as mg per g dry weight with standard curve of glucose (Nelson, 1944). For soluble protein content (Lowry et al., 1951) buffer extract of dry seedlings treated with reagent C(50 ml of A containing 2% sodium carbonate in 0.1 N aqueous NaOH was mixed with 1 ml of B containing 0.5% copper sulphate in 1%, Na-K tartarate) and Folin and Ciocalteau phenol reagent. Amount of soluble protein calculated by standard curve of Bovin Serum Albumin and values expressed as mg per g dry weight. The findings presented are mean of three independent determinations. These are analyzed statistically by standard deviation and checked whether values are significantly different (at P < 0.01) from population or not.

RESULTS AND DISCUSSION

Seed germination and seedling growth

The perusal of data reveals that seed germination in mung bean was inhibited due to treatment of leaf leachate of *D. regia*

(Table 1). Highest inhibition of seed germination was observed at 20% leaf leachate (73.33%), 0.2% (44%) and 0.1% (36%) after 24hrs stage compared to 48hrs (37.66%, 15.58%, 25.97%) and 72hrs (40%, 16.25%, 12.5%) of treatment. Seedling growth with respect to root length and shoot length was reduced due to treatment of leaf leachate at all concentrations in petriplate (Table 2). Reduction in root length was by 74.62%, 17.20% and 47.31% at 20%, 0.2% and 0.1% leaf leachate and 76.43%, 16.98% and 46.70% inhibition in shoot length was observed. In soil bioassay leaf leachate caused 16.09%, 13.73% and 1.02% decline in root length and 9.70% and 7.24% inhibition of shoot length (Table 3).

Starch, sugar and protein contents

In mung bean seedlings, starch content decreased by 14.55%, 10.97% and 36.42% in petriplate bioassay and increased by 42.24%, 1071.12% and 859.66% in soil bioassay. Relative to control, highest elevation in starch content was observed due to 0.2% (1071.12%) and 0.1% (859.66%) in soil bioassay (Table 4). Content of reducing sugar was reduced by 60.94%, 67.45%, 17.45% and 32.93%, 3.44%, 15.17% in petriplate and soil bioassay respectively (Table 5). In mung bean seedlings total sugar content was decreased (43.50%, 57.62% and 18.25%) in petriplate bioassay while it was increased (20.06%, 63.78% and 23.81%) in soil bioassay (Table 6) However reverse trend was observed in soluble protein content, which increased (54.26%, 13.42%, 42.61%) in petriplate and decreased (3.64%, 0.298%) in soil bioassay (Table 7).

Chemical analysis of leaf leachate

Appreciable amount of nitrate and polyphenols were detected in leaf leachates. Camphene, 2-Methyl-2-hepten-6-one, (1S)-2,6,6-Trimethylbicyclo(3.1.1) hept-2-ene, 1R-alpha-pinene, Ocimene, Dimethyloctatriene, Alpha-Fenchene, 4- Nonanone, 7- Methyl-4-octanone, 2-Methyl-3-octanone, 2, 6-Dimethyl-3-Linalol, 2,4,6-Trimethyl-3-Cyclohexen-1heptanone. Carboxyaldehyde, Artemiseole, 2-Isopropenyl-5-methylhex-4enal, (E)-3(10)-Caren-2-ol, Cis-Geraniol, Alpha-citral, Betacitral, Neryl acetate, Caryophyllene, Germacrene, Copaene phytochemicals were detected in leaf leachate (Table 8). Seed is an important organ and germination of seed compiles acute step in propagation and cultivation of most crop species. Seed germination and seedling growth are the imperative morphological parameters studied when plants are exposed to allelochemicals (Kruse et al. 2000). In present study, seed germination is hindered and seedling growth is declined due to leaf leachates (20%, 0.2% and 0.1%) of D. regia. Inhibition was more prominent in 20% leaf leachate treatment. The present findings are in accordance with earlier reports in which evaluation of allelopathic potential of aqueous extracts or aqueous leachates of plant species was carried out by using mung bean as accessible bioassay material. Patil et al. (2013) have examined effect of leaf extract (5%, 10%, 15% and 20%) of Alternanthera tenella, Croton bonplandianum and Xanthium indicum on germination and seedling growth of mung bean by using petriplate bioassay and found reduction in germination percentage due to leaf extract of all the weed species except 10% leaf extract of A.tenella. Complete inhibition of seed germination was reported due to leaf extract of C. bonplandianum (15%, 20%) and X. indicum (20%). Root length and shoot length of mungbean seedlings completely restrained due to high concentration of leaf extract of Croton and Xanthium (20%). As per the observations of Shruthi et al.

(2014) seed germination, radicle and plumule length were reduced due to aqueous extract of powder of dried leaves of Azadirachta indica having 10%, 15% and 20% concentrations in soil bioassays. In existent observation also 20% leaf leachate was initiated as more effective as compare to 0.2% and 0.1%. Maiti et al. (2010, 2013 and 2015) have assessed allelopathic potential of aqueous leaf leachates (1:1, 1:2 and 1:3 proportions) of Lantana camara, Eupatorium odoratum and Hyptis suaveolens on germination of mung bean in petriplate and soil bioassays and reported impediment of seed germination due to treatment of leachates with maximum inhibition at high concentration (1:1). Ojha et al. (2013) noticed inhibition of seed germination of mung bean due to leachates of dry leaves of Melaleuca leucadendron (1:1, 1:2w/v). In mung bean germination percentage was inhibited due to aqueous extract of dried, ground leaves of Moringa oleifera of 2.5%, 5.0%, 7.5%, 10%, 12.5% and 15% concentrations on 3^{rd} , 5^{th} and 7^{th} day of germination in petriplate bioassay (Hossain et al., 2012). Joshi et al. (2015) found decreased radicle length of Vigna radiata in petriplate bioassay by applying 5ml of extract of grinded dry leaves of Ipomoea carnea (2%, 3%, 5%) and Ricinus communis, Hyptis suaveolens, Malachra capitata, Cymbopogon citrulus, Alternanthera sissilis at concentrations 1%, 2%, 3% and 5% and same pattern of inhibition of plumule length due to all the weed species.

Starch forms the major reserve food in many seeds. During germination it is converted to soluble from and transported to the obligatory sites for further metabolism and plant growth. It is involved in regulation of osmotic potential of cells. Sugars are casting building blocks for amino acids and fatty acids. Leguminous seeds are the rich source of proteins, the main source of amino acids for growing embryo. The released amino acids are utilized to construct necessary enzymes and components for seedling growth. In present study starch and sugar contents are decreased in petriplate bioassay and increased in soil bioassay except reducing sugar which is declined due to leaf leachate treatment. On the contrary protein content of mung bean seedlings is increased in petriplate bioassay while decreased in soil bioassay. The present observations corroborate with the earlier findings. Singh and Singh (2003) have observed increase in sugar content of Vigna radiata due to aqueous leaf leachates of Eucalyptus citriodora. Shruthi et al. (2014) found reduction in total carbohydrate content and protein content in shoot and root of mung bean and increase in the same in cotyledons due to 10%, 15% and 20% aqueous extracts of dried leaves of Azadirachta indica in soil bioassay. Maiti et al. (2010) noted increase in soluble carbohydrate content and decrease in protein content in mung bean seeds due to aqueous leaf leachates of Lantana camara (1:1,1:2 proportions) maximum at 1:1 proportion. In extant observations seed germination and seedling growth of mung bean declined and contents starch, sugars and protein are due to leaf leachate treatment higher at 20% by altered following similar petriplate and soil bioassays and applying leaf leachates for treatment and distilled water for control. In present investigation phytochemicals detected in leaf leachates of D. regia might be involved in inhibition of seed germination, seedling growth and altered biomolecules are in compliance with earlier reports on camphene, alpha-pinene, ocimene, nonanone, copaene and caryophyllene. Nishida et al. (2005) noted inhibition of cell proliferation, DNA synthesis in root apical meristem and root growth of Brassica campestris seedlings due to camphene and alpha-pinene isolated from

Salvia leucophylla. Alpha- pinene and Copaene isolated and identified from Eucalyptus urophylla by Qui et al. (2010). Xue et al. (2011) observed inhibition of germination and seedling growth of Levmus chinensis, Stipa krylovii and Cleistogenes squarrosa due to camphene and alpha-pinene isolated from Artemissia frigida. Kanchiswamy et al (2015) reported production of camphene, alpha-pinene, ocimene, nonanone, copaene and caryophyllene by a wide array of microorganisms from bacteria to fungi. Phytochemicals active as an allelochemicals may manipulate growth and development by inhibiting root elongation, cell division, changing cell ultrastructure, reducing chlorophyll content and photosynthetic rate, declining enzyme activities and protein synthesis (Zhao-Hui et al., 2010). Accumulation of nitrate in soil, due to reduction in root surface and number of root nodules, causes hostile effects on nitrogen fixation in legume plants (Hannaway and Shuler, 1993). Serious health disorders have been observed in animals due to consumption of plants with high nitrate content (Shiel et al. 1999). Inhibition of seed germination and reduced seedling growth due to leaf leachate at high and low concentration may further affect the growth and development of V. radiata. In soil bioassay seedling growth was not adversely affected as in petriplate bioassay, which might be due to degradation of allelochemicals by soil microbes or by binding of allelochemicals to soil particles. Disturbed metabolism of Vigna due to alterations in the contents of starch, sugars and proteins, may obstruct growth of seedlings which may affect further growth, development and yield of crop plant. Reduction in seed germination and seedling growth of mung bean may be synergistic effect of the appreciable amount of polyphenols and phytochemicals detected in leaf leachate. Growth performance of mungbean under allelopathic potential of Gulmohar may help to select plant species in agroforestry programme. Chemical analysis of leachate may be utilized for selection of phytochemicals for eradication of weeds.

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