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SEASONAL VARIATION OF AMINOACIDS AND CARBOHYDRATES IN HAEMOLYMPH OF SPODOPTERA LITURA FABRICIUS (LEPIDOP TERA: NOCTUIDAE) FED WITH DIFFERENT HOST PLANTS

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Abstract:
Spodoptera litura, an important polyphagous pest, causes serious threat to a large number of economically important crops all over the world. In the present study, the quantitative analysis of aminoacids and carbohydrates in haemolymph of the last instar larvae of Spodoptera litura fed with five different host plant leaves was carried out. A comparative study of the biochemical components in the selected host plant leaves was also carried out. The data obtained were statistically analysed using one-way ANOVA (P<0.01). The results revealed that there was a significant variation in the concentration of these biochemical components in haemolymph with respect to the feeding material and with seasonal variation.

Keywords: Spodoptera Litura; Host Plants,Haemolymph; Amino Acid And Carbohydrate Concentration.

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1. Introduction

Spodopteralitura is one of the economically important polyphagous lepidopteran pest [1]. The present work deals with the biochemical analysis of haemolymph of Spodoptera litura during the last larval instar development. The food quality is very important for the growth, development and reproductive potential which depends mainly on nutritional composition, including both the absolute and relative amount of water, proteins, amino acids, carbohydrates, lipids, minerals etc. Carbohydrate plays a significant role in determining the leaf quality which in turn influences the growth and development of the phytophagous insects. The amount of dietary prerequisite of carbohydrate in insect larvae differ variably. The ingested carbohydrates together with nutrient reserve carried over from the larval stage are important to meet the energy demand of the insects [2].

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Haemolymph, which is the only extra cellular fluid in insects, has diverse functions, such as immunity, transport and storage of the products required for cellular metabolism. Investigations on insect haemolymph are of particular interest because they provide us an adequate background to judge the synthetic activity associated with the different processes in developing organisms. The haemolymph plasma contains almost all inorganic constituents like electrolytes or ions, phosphates and organic constituents like free amino acids, proteins, lipids, carbohydrates, uric acid etc.The chemical composition of haemolymph is highly variable among the species and at different developmental stages of the same species. Several factors such as diet, diseases and temperature influence the insect haemolymph composition of a species [3]. Various changes also occur in the biochemical composition of haemolymph during metamorphosis. These changes reflect the morphogenic and biochemical changes in the body of the insect, in response to ambient environment. Studies on quantitative and qualitative analysis of haemolymph in various insects like Drosophila, Periplaneta, Calliphora, Bombyx mori and other lepidopterans have been reported. In the present study, investigations have been carried out to evaluate the quantitative changes in the concentration of amino acid and carbohydrate in the haemolymph of last instar larvae of Spodoptera litura fed with selected host plant leaves during different seasons.

2. Materials and Methods

2.1. Insect Culture

A culture of S.litura was established under laboratory conditions from the pupae obtained from the insect rearing centre ICAR Bangalore. The pupae were kept in a plastic tray and placed it in a wooden cage covered with thin gauze. As soon as the adult emerged, they were transferred into plastic containers covered with muslin cloth and allowed them to mate. Absorbent cotton ball soaked in 20 % honey-water was provided for the adult during the oviposition period. The eggs laid by the adult along the sides of the plastic containers were kept for incubation. Hatched larvae were transferred to the plastic trough and were fed with fresh host plant leaves. 25-30 larvae were cultured in each trough. Each day the leaves were replaced with fresh one. The utilization of the host plant leaves and the larval growth and development was observed. The last instar larvae from the second generation were used for the experiment.

2.2. Host Plants

Five host plants selected in this study were castor (Ricinus communis), colocasia(Colocasia esculenta) and papaya(Carica papaya), banana(Musa accuminata Colla) and sweet potato(Ipomea batata).These plants were selected because they are the primary host plants of S. litura and are important as economic crops.

2.3. Collection and Preparation of Tissue Sample

Amino acid and carbohydrate estimation was carried out in the haemolymph of last instar larvae of S.litura fed with five different host plant leaves in three different seasons-summer, monsoon and post monsoon. Five replicates containing 0.2ml ofhaemolymph from single larva were maintained for each analysis.

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2.3.1. Haemolymph

The larvae were anesthetized and the haemolymph was collected by amputing the legs using a calibrated capillary tube. The haemolymph was deproteinized using 80% ethanol and centrifuged at 10,000rpm for 10 minutes. Supernatant was used for the estimation of amino acid and carbohydrates.

2.3.2. Preparation of Leaf Tissue

500mg of the leaf tissue were weighed and ground it in a pestle and mortar with a small quantity of acid washed sand. 5-10ml of 80% ethanol was added to this homogenate and centrifuged. Saved the filtrate. Repeated the extraction twice with the residue and collected the supernatants and these extracts were used for the estimation of total free amino acids.

100mg of the leaf tissue was weighed in to a boiling tube and it was hydrolysed by keeping it in a boiling water bath for three hours with 5ml of 2.5N HCl and cooled to room temperature. It was neutralized with solid sodium carbonate until the effervescence ceased. Then the volume was made up to 100ml and centrifuged. The supernatant was used for the estimation of total carbohydrates.

2.4. Estimation of Aminoacid and Carbohydrate

Quantitative estimation of aminoacid in the haemolymph samples and in the leaf tissue samples were done spectrophotometrically by the Ninhydrin method [4] and that of carbohydrate by Anthrone method [5].

2.5. Statistical Analysis

One -way analysis of variance (ANOVA) was used to test the significance of differences between mean values of amino acid and carbohydrate content in the haemolymph of last instar larvae of S.litura fed with different host plant leaves. Comparisons were performed to find out the significant difference in the amino acid and carbohydrate concentration in the haemolymph depending on the feeding rate of different host plant leaves. The difference was significant at $p < 0.01$.

3. Results and Discussion

3.1. Seasonal Variation in Amino Acid Concentration in Leaves of Selected Host Plants and Haemolymph of Spodoptera Litura

The total free aminoacid concentration in five selected host plant leaves in three different seasons were shown in the Table.1 and Figure.1. Changes in the amino acid concentration of selected host plant leaves in different seasons were noticed. Among the five selected host plants, the castor leaves showed the highest amino acid content of 26.7±0.17 mg/ml. The amino acid concentration in other host plant leaves were 25.8±0.47mg/ml (papaya), 24.4±0.19mg/ml (colocasia), 23.4±0.34mg/ml (banana) and 22.8±0.17mg/ml (sweet potato) respectively in summer season. Similar changes were noticed in other seasons also. Similarly the concentration of amino acid in the haemolymph (Table.2 and Figure.2) of the larvae fed with different host plant leaves showed

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increase in the order castor>papaya>colocasia> banana>sweet potato. In all the three seasons the variation in the amino acid concentration in the haemolymph was similar. The statistical analysis of the data showed significant difference in the amino acid content of both haemolymph and host plant leaves in each season.

Table 1: Seasonal variation in amino acid content in the leaves of selected host plants.

Seasons		Amino acid concentrati on in	Host plants					F	P
			Castor	Papaya	Colocasi a	Banana	Sweet potato		
	Summer	Mg/ml	28.7±0.1	25.8±0.4	24.4±0.1	22.4±0.3	20.8±0.1	28.	0.00
		Mg/gm	0.53±0.0	0.51±0.0	0.49±0.0	0.47±0.0	0.46±0.0	28.	0.00
	Monso	Mg/ml	24.1±0.5	23.2±0.2	22.7±0.1	19.4±0.1	18.8±0.1	9.5	0.00
		Mg/gm	0.48±0.0 1	0.45±0.0 0	0.40±0.0 0	0.36±0.0 0	0.29±0.0 0	9.5	0.00 0
	Post	Mg/ml	22.8±0.5	20.3±0.0	19.4±0.0	18.4±0.5	15.1±0.4	27.	0.00
		Mg/gm	0.45±0.0 1	0.40±0.0 0	0.39±0.0 0	0.34±0.0 1	0.24±0.0 0	27. 7	0.00 0

The values presented in the table are the mean value of five replicates for each host plant leaves with standard error (Mean ±Standard error). All the values were found to be significantly different at 1% level. The total amino acid content was expressed in mg/ml and mg/gm tissue.

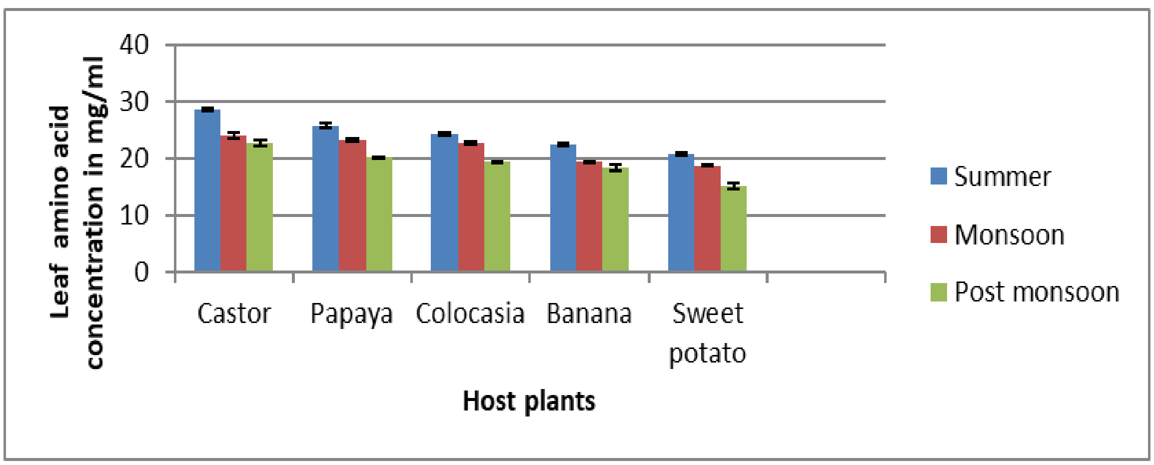

Figure 1: Seasonal variation in the amino acid content of the selected host plant leaves.

Table 2: Seasonal variation in aminoacid content of the haemolymph of last instar larvae of Spodoptera litura after fed with the leaves of selected host plants.

Seasons	Summer	Aminoacid concentrati on in	Host plants					F	P
			castor	papaya	Colocasi a	banana	Sweet potato		
		Mg/ml	35.6±0.8 6	34.5±0.8 9	33.1±2.4 6	29±1.8	20.9±0.7 5	20.0 8	0.00 0
		Mg/insect	7.12±0.1 7	6.91±0.1 7	6.63±0.4 9	5.8±0.36	4.19±0.1 5	15.4	0.00 0

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	Monsoon	Mg/ml	34.1±1.2	33.1±0.39	30.2±2.5	27.1±0.97	18.7±0.46	20.08	0.000
		Mg/insect	6.8±0.24	6.6±0.07	6.5±0.27	5.4±0.19	3.7±0.09	43.5	0.000
	Post	Mg/ml	32.3±0.27	30.5±1.1	28±2.7	26±1.2	17.4±0.66	15.4	0.000
		Mg/insect	6.4±0.05	6.1±0.22	5.6±0.55	5.1±0.24	3.5±0.13	15.4	0.000

The values presented in the table are the mean value of five replicates for each host plant with standard error (Mean ±Standard error). All the values were found to be significantly different at 1% level. The total amino acid content was expressed in the mg/ml and mg/insect haemolymph.

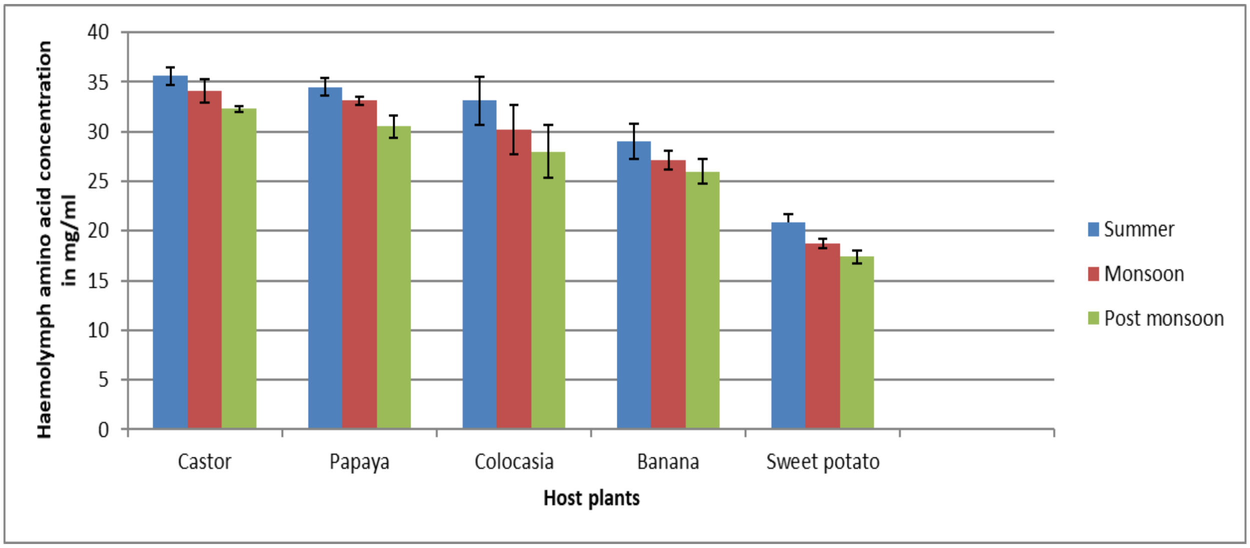

Figure 2: Seasonal variation in amino acid content of the haemolymph of last instar larvae of Spodoptera litura after fed with the leaves of selected host plants

3.2. Seasonal Variation in Carbohydrate Concentration in Leaf Tissues of Selected Host Plants and Haemolymph of Spodoptera Litura

Variation in the total carbohydrate content in the leaves of five selected host plants (Table.3 and Figure.3) and in the haemolymph of last instar larva of Spodoptera litura (Table.4 and Figure.4) in different seasons were noticed. Variation of carbohydrate content in each host plant leaf was noticed in each season. Comparison of the results obtained from the data indicated that from the five different host plants selected the castor showed higher carbohydrate content than the other four host plants and it contained 0.037±0.00mg/ml of carbohydrate. The carbohydrate content in other host plants papaya, colocasia, sweet potato and banana are in the order 0.036±0.00mg/ml, 0.034±0.00mg/ml, 0.033±0.00mg/ml and 0.028±0.0mg/ml respectively in summer season. Similar changes were noticed in monsoon season and post monsoon season. By comparing the seasonal variation it was observed that in the summer season all the selected plant leaves showed the highest carbohydrate content than in the monsoon and post monsoon seasons.

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Table 3: Seasonal variation in carbohydrate content of selected host plant leaves.

Seasons		Carbohydrate concentration in	Host plants					F	P
			Castor	Papaya	Colocasia	Sweet potato	Banana		
	Summer	Mg/ml	0.037±0.00	0.036±0.00	0.034±0.00	0.033±0.00	0.028±0.0	0.936	0.463
		Mg/gm	0.37±0.00	0.36±0.03	0.34±0.01	0.33±0.01	0.28±0.00	0.657	0.629
	Monsoon	Mg/ml	0.032±0.00	0.030±0.00	0.027±0.00	0.027±0.00	0.025±0.0	7.80	0.001
		Mg/gm	0.32±0.00	0.30±0.00	0.27±0.01	0.27±0.01	0.25±0.00	7.77	0.001
	Post	Mg/ml	0.029±0.00	0.021±0.00	0.020±0.00	0.013±0.00	0.007±0.0	3.59	0.023
		Mg/gm	0.29±0.08	0.21±0.01	0.20±0.00	0.13±0.01	0.07±0.03	3.59	0.023

The values presented in the table are the mean value of five replicates for each host plant leaves with standard error (Mean ±Standard error). All the values were found to be significantly different at 1% level. The total carbohydrate content was expressed in mg/ml and mg/gm tissue.

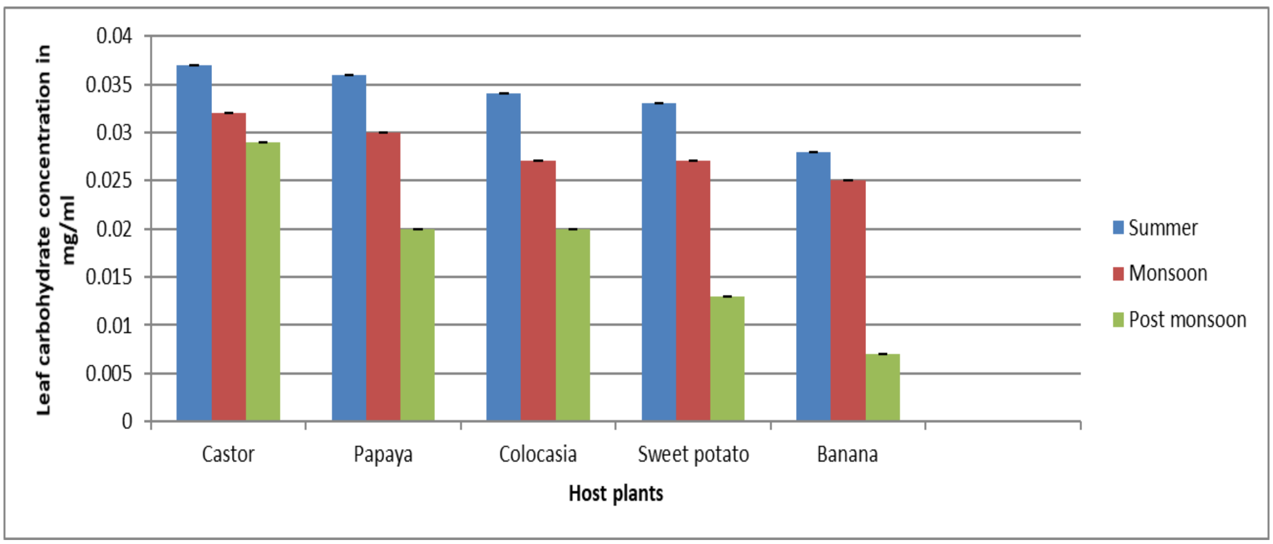

Figure 3: Seasonal variation in carbohydrate content of selected host plant leaves

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Table 4: Seasonal variation in carbohydrate content of the haemolymph of last instar larvae of Spodoptera litura after fed with the leaves of selected host plants

Seasons		**Carbohydrate concentration in**	**Host plants**					**F**	**P**
			Castor	**Papaya**	**Colocasia**	**Sweet potato**	**Banana**		
	Summer	Mg/ml	1.8±0.01	1.7±0.04	1.4±0.08	1.2±0.02	0.78±0.03	71.5	0.000
		Mg/insect	0.36±0.00	0.34±0.00	0.27±0.01	0.25±0.00	0.17±0.01	50	0.000
	Monsoon	Mg/ml	1.5±.07	1.1±0.06	0.89±0.03	0.84±0.03	0.67±0.02	43.8	0.000
		Mg/insect	0.25±0.06	0.24±0.01	0.22±0.01	0.19±0.00	0.16±0.00	1.5	0.245
	Post	Mg/ml	1.3±0.06	0.70±0.05	0.53±.00	0.47±0.02	0.24±0.00	100	0.000
		Mg/insect	0.27±0.00	0.14±0.01	0.09±0.00	0.07±0.00	0.05±0.00	220	0.000

The values presented in the table are the mean value of five replicates for each host plant with standard error (Mean ±Standard error). All the values were found to be significantly different at 1% level. The total carbohydrate content was expressed in mg/ml haemolymph and mg/insect.

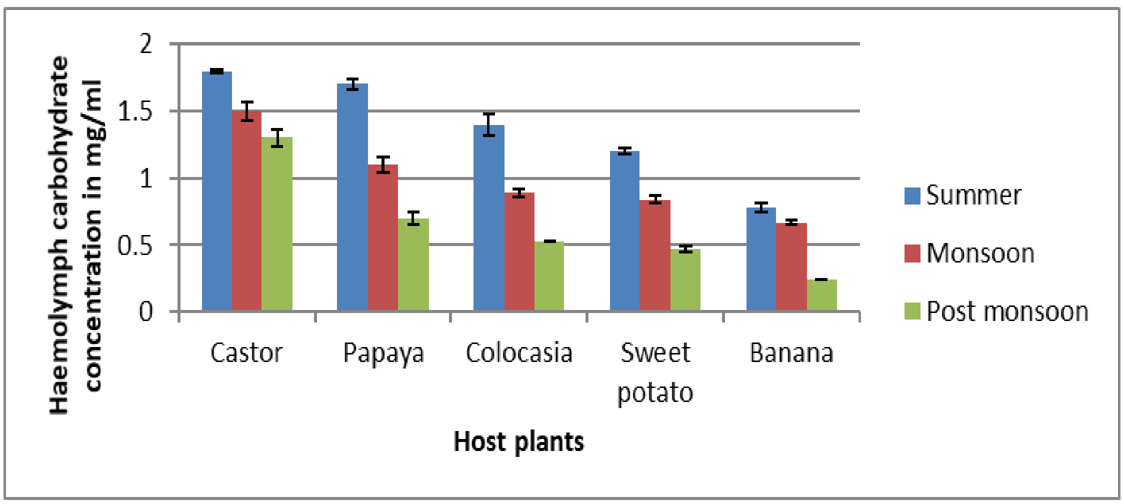

Figure 4: Seasonal variation in carbohydrate content of the haemolymph of last instar larvae of Spodoptera litura after fed with the leaves of selected host plants

The results of the present study revealed that there was considerable variation in the amino acid and carbohydrate content in haemolymph of the last instar larvae of S. litura fed with selected host plant leaves during different seasons. This may be due to the significant difference in the nutritional value of the host plant leaves with changing seasons. The quality of an herbivore insect diet changes both within and between its host plants and these fluctuations can be predictable, such as seasonal changes in plant quality or unpredictable, such as the changes caused by environmental stress [6].

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Among the selected host plants the castor leaves showed highest amount of amino acid and carbohydrate followed by papaya, colocasia, sweet potato and banana. The present observations were in agreement with the findings of previous works of many researchers. According to them, for plants changes in carbohydrate content existed at a number of levels, including between the species [7], within the species [8] and within an individual plant [9] depending on the type of tissue (i.e., leaves, flowers, seeds and stems) and its age (i.e., young versus old leaves). Besides, a plant's protein and carbohydrate content can vary in response to environmental factors including the amount of light it receives, the chemical composition of the soil and inputs of water [10,11].

Similarly, the larval haemolymph showed the highest content of amino acid and carbohydrate during the summer season than the monsoon and post monsoon seasons. This variation may be either due to the fluctuations in temperature or humidity or by changes in any other environmental factors. Various studies done on numerous insect species revealed that temperature rise lead to increased metabolic rate [12,13]. This finding is supported by the work of Mullins [14] that various factors like temperature and diet influence the composition of the insect tissues.

The present study revealed the effect of dietary components on the biochemical composition of larval haemolymph. Seasonal variation in biochemical components was seen in different host plant leaves. The quality of host plants determines the tissue components of the insects (eg., the levels of protein, carbohydrate, trace elements and defensive compounds) that can affect the feeding and digestion positively or negatively in the herbivorous insects [15].

4. Conclusion

The findings revealed a positive correlation between the amino acid content and carbohydrate content in the plant leaves and in the haemolymph of the larvae feeding on the specific plant materials. Among the selected host plants, castor leaves showed highest content of amino acid and carbohydrate which was found to be correlated with a corresponding increase in content of amino acid and carbohydrate concentration in the haemolymph of the larvae fed with castor leaves. Generally, the concentration of amino acid and carbohydrate were found to be reduced in the tissues of the larvae fed with sweet potato with the corresponding reduction in these biochemical content in host plant leaves. These findings reveal that variation in biochemical components of host plant leaves can influence the biochemical components in insect tissues during different seasons.

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