Allelopathic effect of *Rhazya stricta* plant residue on carbohydrate contents of *Raphanus sativus* (Radish) of 30-day-old

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الملخص:

تمت إجراء تجربة البيت الزجاجي لتقييم التأثيرات الأليلوباثية لنبات الحرمل(*Rhazya stricta*) علي محتويات الكربوهيدرات للفجل في عمر 30 يوم. لم يتأثر المجموع الكلي للسكريات القابلة للذوبان بشكل معنوي. أظهر مسحوق نبات الحرمل آثارا سلبية علي الكربوهيدرات غير القابلة للذوبان ومحتوي الكربوهيدرات الكلية لنبات الفجل خاصة عند التركيزات العالية.

كلمات مفتاحية: المواد الأليلوكيميائية، مسحوق، كربوهيدرات، أليلوباثي، نبات الحرمل.

ABSTRACT:

A greenhouse pot experiment was conducted to assess the allelopathic effects of *Rhazya stricta* plant residue on carbohydrate contents of 30-day-old radish (*Raphanus sativus*). The total soluble sugar were not significantly affected. The residue of *Rhazya* showed negative effects on insoluble carbohydrate and total carbohydrate contents of *R. sativus* particularly at the high concentration.

Keywords: allelochemicals, residue, carbohydrate, allelopathy, *Rhazya stricta*.

Introduction

Allelopathy is the direct influence of chemicals released from one plant on the development and growth of another plant (Olofsdotter. 1998). Plants produce a large number of chemical compounds which vary in their chemical com- positions and concentrations. The protein, carbohydrate and chlorophyll contents of plants are considerably influenced by allelochemicals (Kohli. 1987). In arable ecosystems, plants growth and development is associated with their interaction. Plants directly affect their development through their competition to acquire the environmental nutrient elements or indirectly they made this inhibition with their radicular leakage, obtained distillates of growing residues and other soil incorporated residues (Golzardi *et al.* 2015). Allelopathy refers to the beneficial or harmful effects of one plant on another plant, both crop and weed species, by the release of chemicals from plant parts by leaching, root exudation, volatilization, residue decomposition, and other processes in both natural and agricultural systems (Khan *et al*, 2011).

Rhazya stricta Decne, (Apocynaceae) is one of the dominant species in the rangeland of Saudi Arabia. Through allelopathic activity, it has been shown to inhibit seedling growth (Assaeed and Al- Doss 1996). This phenomenon has encouraged the search for and the development of natural products that have pest-controlling activities (Chou. 1999). Several studies found that alkaloidal extracts from plants have herbicidal activity (Wink. 1983, Petroski *et al.* 1990, Elakovich and Yang. 1996). *R. stricta* contains many alkaloidal compounds (Hassan *et al.* 1977) that are able to affect the growth of pests such as

Culex pipiens (El-Hag *et al*. 1996), nematodes e.g. Meloidogyne javanica (Al-Rajhi *et al*. 1997) and weeds (Al-Mutlaq *et al*. 2002). (Al-Yahya *et al*.1990) have reported the presences of alkaloids, glycosides, triterpenes, tannins and volatile bases in the leaves of this plant.

Raphanus sativus (radish) is a globally edible root and leaf vegetable. Radish is rich in ascorbic acid, folic acid, and potassium. It is also a good source of vitamin B6, riboflavin, magnesium, copper and calcium, *Raphanus sativus* contains flavonoids, saponins, tannins, glycosides, steroids and alkaloids. (Abad. 2019). The objective of the present study was conducted to investigate the allelopathic effects of *R. stricta* plant residue on carbohydrate contents of *R. sativus* of 30-day-old.

Material and Methods

Plant materials

Plant material of *Rhazya stricta* was collected from its natural habitats in central Saudi Arabia. The plants were air dried, then ground into a fine powder and stored in refrigerator until used. The seeds of radish were obtained from the Agricultural Research Center, Vegetables Department, Egypt.

Pot experiment

A greenhouse pot experiment was conducted to assess the possible inhibitory or stimulatory effects of *Rhazya* plant powder on *Raphanus sativus* plant. Pot experiment was carried out in plastic pots (13 cm in diameter and 14 cm in depth), each containing 2 kg of clay soil. The pots were divided into 8 groups, each was 12 pots, one was left without treatment as control and the other seven groups were treated with *Rhazya* residues. The fine ground shoot powder was incorporated into the upper soil layer with 2 cm depth that finally gave the percentages of 2, 4, 6, 8, 10, 12 and 16% (w/w). Ten healthy *R. sativus* seeds of uniform size were sown at 1 cm soil depth and the seedlings were thinned to 5 plants per pot after emergence. Plants were irrigated with tap water, and soil was kept at field capacity, along the whole experimental period, using weighing procedure. Pots were placed in an open greenhouse under natural conditions during March to April months. The plants, at the vegetative stage, were harvested after 30 days from sowing, then washed thoroughly with tap water and were oven dried to a constant weight at 80^{o}C for dry weight measurements.

Extraction and Determination of Carbohydrate

1- Extraction of soluble sugar

Soluble sugars were extracted from the plant tissues according to the method described by (Upmeyer and Koller, 1973). Sample of 0.05 gram powdered dry tissue was homogenized three times with hot 80 % ethanol. The extract was centrifuged at 4000 g for 15 min. then completed up to a known volume with 80 % ethanol. The mixture was shaken for 5 min. and then filtered through filter paper. The decolorized extract was used for the estimation of soluble sugar content (reducing and non-reducing). The plant residue was dried and stored for estimation of insoluble carbohydrate.

2-Hydrolysis of non-reducing sugar

One ml of soluble sugar extract was hydrolyzed with 1 ml 6 N HCl by heating the mixture at 70 ^{o}C in water bath for 12 min. (Gaines, 1973). The mixture was neutralized, then diluted to a known volume with water and its total reducing value was determined.

3- Extraction of insoluble carbohydrate

The dried plant residue remained after extraction of soluble sugar was hydrolyzed with 0.2 N H_2SO_4 by reflux for 1 hour at 100 ^{o}C (Streeter and Jeffers, 1979). The mixture was centrifuged for 15 min at 4000 g. The clear supernatant neutralized then made up to a known volume with 80 % ethanol and its reducing value determined.

4- Determination of reducing value

The reducing value of sugar extract obtained from steps (1), (2) and (3) was estimated by Nelson`s test as described by (Clark and Switzer, 1977). One ml of neutralized sugar extract was mixed with 1 ml of freshly prepared Nelson`s alkaline copper reagent (Nelson`s A and Nelson`s B reagents in ratio of 25:1) and heated in a boiling water bath for 20 min., then rapidly cooled under running water. One ml of arsenomolybdate reagent (25 g of ammonium molybdate dissolved in 450 ml distilled water, 21 ml conc. H_2SO_4, and 3 g sodium arsenate dissolved in 25 ml distilled water) was added with shaking until effervescence stopped. The mixture was made up to a known volume with distilled water. The absorbance of the bluish green colour was measured at wavelength 540 nm against water blank treated as samples. The reducing value was determined from standard curve of glucose and calculated as mg sugar g^{-1} dry weight. The non-reducing sugar content was calculated by subtracting the reducing value of extract before and after hydrolysis.

Statistical analysis

The data obtained were analyzed with (SPSS) one-way ANOVA.

Results

Changes in the carbohydrate fractions of *R. sativus* for 30- days-old with different rates of *Rhazya* plant residue are shown in (Figure & Table1). Generally no significant differences in the reducing soluble sugar of *R. sativus* as result of different *Rhazya* residue concentrations. In case non reducing soluble sugar of *R. sativus* there was no significant effect by different concentrations of *Rhazya* residue except at 6 grams residue concentration, Table (1). With respect to total soluble sugar of *R. sativus* generally showed no constant trend with increase of *Rhazya* residue concentration, Figure (1). In contrast, reducing insoluble carbohydrate of *R. sativus* showed a decrease in insoluble carbohydrate with increase the concentrations of *Rhazya* residue except at 2 grams residue concentration which showed a marked increase in insoluble carbohydrate of the treated plant as shown in (Table &Figure 1). Highest reduction in insoluble carbohydrate of *R. sativus* reached 104.19 mg/g dry weight at residue concentration of 10 grams. The total carbohydrate of *R. sativus* increased *Rhazya* residue caused a significant decline in *R. sativus* total carbohydrate particularly at 10 grams residue concentrations (Table &Figure 1).

Table 1. Effect of different concentrations of *Rhazya stricta* plant residues on various carbohydrate contents of 30 -day-old *Raphanus sativus* plant.

Residue Concentration (g)	Soluble Sugar (mg/g Dry Weight)		Total Soluble Sugar (mg/g Dry Weight)	Insoluble Carbohydrate (mg/g Dry Weight)	Total Carbohydrate (mg/g Dry Weight)
	Reducing	Non Reducing			
0	65.46 ± 0.63[a]	57.71 ± 1.77[ab]	123.17 ± 2.03[a]	120.86 ± 0.8[e]	244.03 ± 1.76[cd]
2	64.85 ± 1.32[a]	57.19 ± 3.05[a]	122.04 ± 4.2[a]	124.94 ± 2.25[f]	246.97 ± 5.73[d]
4	65.48 ± 0.89[a]	60.09 ± 2.6[abc]	125.57 ± 2.41[a]	116.1 ± 1.26[c]	241.67 ± 2.48[bc]
6	64.61 ± 1.77[a]	60.79 ± 2.34[c]	125.4 ± 3.33[a]	113.51 ± 0.98[b]	238.91 ± 2.84[b]
8	65.32 ± 1.29[a]	58.08 ± 2.06[abc]	123.39 ± 2.56[a]	116.84 ± 1.29[c]	240.14 ± 3.64[b]
10	64.64 ± 1.4[a]	59.46 ± 1.26[abc]	124.1 ± 1.83[a]	104.19 ± 1.6[a]	228.29 ± 2.64[a]
12	64.87 ± 1.25[a]	58.39 ± 2.89[abc]	123.25 ± 2.16[a]	115.56 ± 1.21[c]	238.81 ± 1.71[b]
16	64.93 ± 1.18[a]	60.23 ± 0.83[bc]	125.16 ± 1.66[a]	118.84 ± 1.4[d]	243.99 ± 1.02[cd]

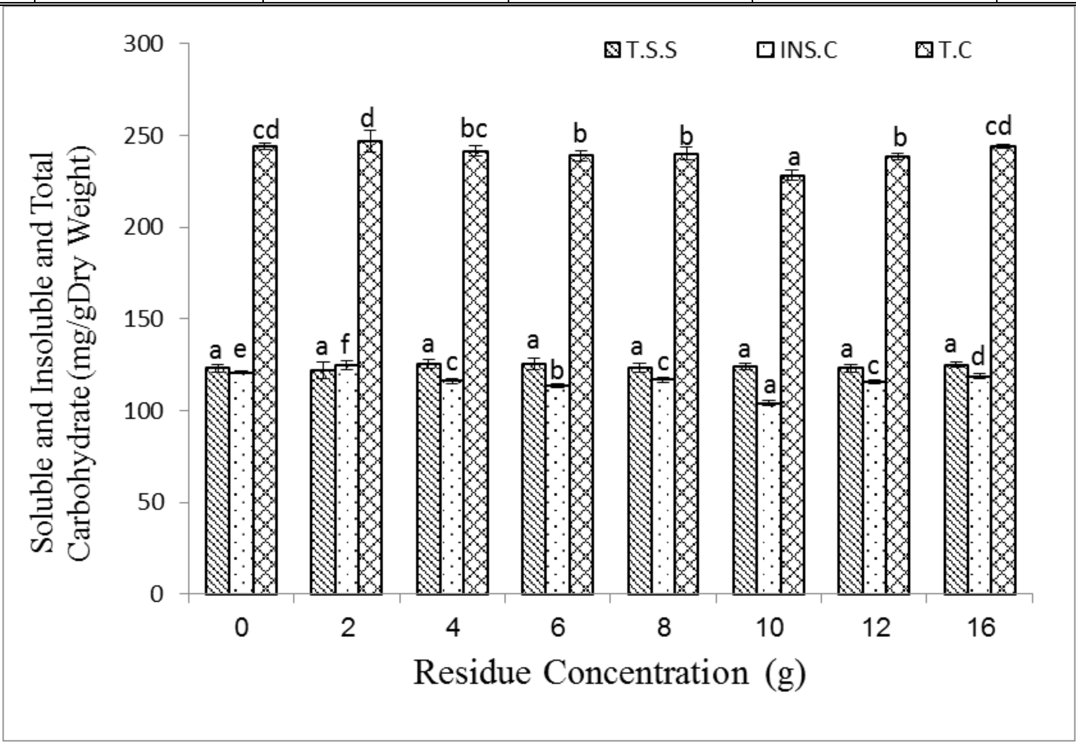

Figure 1. Effect of different concentrations of *Rhazya stricta* plant residues on the carbohydrate content (Total soluble sugar, Insoluble carbohydrate and Total carbohydrate) of 30-day-old *Raphanus sativus*. Vertical bars are standard deviation of the mean.

Discussion

The results of the present study showed that the plant powder of *Rhazya stricta* differed in their effects on carbohydrate contents of Radish (*Raphanus sativus*) plant. The biochemical changes of the shoot of 30-day-old Radish in response to *Rhazya* treatments showed a considerable variation in the amounts of carbohydrate, which represent one of the main organic constituents. The accumulation of total carbohydrate with the 2 grams *Rhazya* treatments was attributed to an increase in the insoluble carbohydrate and a decline in the total soluble sugar. This suggests that lower *Rhazya* residue had no harmful effect on the total carbohydrate and stimulate the conversion of soluble sugar into insoluble form. Inversely, all the carbohydrate fractions were greatly attenuated with the 10 and 12 grams *Rhazya* residue. Similarly, Tripathi *et al.* (1998) showed that the total sugar content in soybean leaves increased with 5 % concentration of *Acacia nilotica* leaf extract, while the 10 and 20 % extracts retarded this accumulation. In other allelopathic study, Al-Wakeel and Soliman. (1994) found that the low concentration of lupin seed extract increased the total carbohydrate content in the shoot of 60-day-old-soybean, while the high concentration reduced this parameter.

Conclusion

The present investigation revealed that *Rhazya stricta* residue contain some substances which have inhibitory effects on *Raphanus. sativus*. Therefore, further studies are recommended to investigate the possible physiological mechanisms related to allelopathic effect on plants.

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