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العدد 23

مجلة التربوي

مجلة علمية محكمة تصدر عن كلية التربية

جامعة المرقب

العدد الثالث والعشرون
يوليو 2023م

- المجلة ترحب بما يرد عليها من أبحاث وعلى استعداد لنشرها بعد التحكيم .
- المجلة تحترم كل الاحترام آراء المحكمين وتعمل بمقتضاها .
- كافة الآراء والأفكار المنشورة تعبر عن آراء أصحابها ولا تتحمل المجلة تبعاتها .
- يتحمل الباحث مسؤولية الأمانة العلمية وهو المسؤول عما ينشر له .
- البحوث المقدمة للنشر لا ترد لأصحابها نشرت أو لم تنشر .

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يشترط في البحوث العلمية المقدمة للنشر أن يراعى فيها ما يأتي :

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- ألا تكون المادة العلمية قد سبق نشرها أو كانت جزءا من رسالة علمية .
- يرفق بالبحث تزكية لغوية وفق أنموذج معد .
- تعدل البحوث المقبولة وتصحح وفق ما يراه المحكمون .
- التزام الباحث بالضوابط التي وضعتها المجلة من عدد الصفحات ، ونوع الخط ورقمه ، والفترات الزمنية الممنوحة للتعديل ، وما يستجد من ضوابط تضعها المجلة مستقبلا .

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- يخضع البحث في النشر لأولويات المجلة وسياستها .
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A comparative study of the effects of *Rhazya stricta* plant residue on *Raphanus sativus* plant at the age of 15 and 30 days

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Abstract: A greenhouse pot experiment was conducted to assess the effects of *Rhazya stricta* plant residue on *Raphanus sativus* plant. The residue of *R. stricta* showed inhibitory effect on root length, dry weight and root to shoot length ratio of *R. sativus* especially at the high concentrations at different ages. The *Rhazya* residue showed positive effects on the photosynthetic pigments of *R. sativus* particularly on the carotenoids and chlorophyll a/b ratio at different ages. A significant increase in nitrogen content of *R. sativus* including total amount of free amino acid, soluble and insoluble nitrogen and crude protein was prominent at the late growth stage especially at the high concentrations. The *Rhazya* residue inhibited the contents of nitrogen of *R. sativus* in the early growth stage.
Keywords: allelochemicals**,** residue, photosynthetic pigments, nitrogen, growth.

الملخص

تمت دراسة تأثيرات مسحوق نبات الحرمل (*Rhazya stricta*)علي الفجل تحت الظروف الطبيعية. أظهر مسحوق نبات الحرمل تأثيرا مثبطا علي طول جذر النبات والوزن الجاف إضافة إلي نسبة طول الجذر/ الساق خصوصا في التركيزات العالية وفي مختلف الأعمار. كما أظهر مسحوق الحرمل آثار إيجابية علي أصباغ البناء الضوئي لنبات الفجل خاصة الكاروتينات ونسبة الكلوروفيل a/b عند مختلف الأعمار. وقد ارتفع محتوي النبات من الأحماض الأمينية الحرة ومحتوي النيتروجين القابل وغير القابل للذوبان والبروتين بنسب واضحة في مراحل النمو االمتأخرة عمر(30 يوم) خاصة في التركيزات العالية، بينما انخفض محتوي النبات من النيتروجين القابل للذوبان وكان ذلك في مراحل النمو المبكرة (عمر 15 يوم).

الكلمات الدالة: المواد الأليلوكيميائية، مسحوق ، أصباغ البناء الضوئي، نيتروجين، نمو.

Introduction

Allelopathy can be defined as the effects of one plant species on another plant species (mostly harmful effects) through the release of chemical compounds (allelochemicals) that escape into the environment. Plants can release allelochemicals into the environment via root exudation, leaching by rains, or decomposition of plant residues. Moreover, evidence exists for airborne allelopathy mediated by volatile allelochemicals. Allelochemicals are present in almost all plants and their tissues such as leaves, stems, roots, flowers, seeds bark and buds. Allelopathy is currently practiced in organic agriculture as a biological mean to control weed instead of using herbicides (Abu-Romman., 2011, p. 947).

Rhazya stricta Decne, an evergreen poisonous shrub of the Apocynaceae family is a wild plant widely distributed in the sandy plains of Saudi Arabia and comparable

habitats throughout the world (Baeshin *et al*. 2005). *R. stricta* contains many alkaloidal compounds that are able to affect the growth of pests such as *Culex pipiens*, nematodes e.g. *Meloidogyne javanica* and weeds (Al-Mutlaq 2001& Al-Mutlaq *et al.* 2002). In previous studies, (Al-Mutlaq., 2001 & Al-Mutlaq *et al.* 2002) reported that *Rhazya stricta* leaf leachates and total alkaloidal extracts affected seedling development of alfalfa (*Medicago sativa*), wild radish (*Raphanus sativus*) and Italian ryegrass (*Lolium multifl orum*) but not wheat (*Triticum aesti- vum*). *R. stricta* like other plants is competing with the main crops for nutrients and other resources and hamper the healthy growth of crops ultimately, reducing the yield both qualitatively and quantitatively (Abad ., 2022).

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 (Jan & Badar., 2012, p. 23). Thus the aim of this study was conducted to explore the effects of *R. stricta* residue on *R. sativus* plant in greenhouse pot experiment at the age of 15 and 30 days.

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 month. The plants, at the vegetative stage, were harvested after15 and 30 days from sowing, then washed thoroughly with tap water and divided into root and shoot systems for measurement of growth criteria. Lengths of the main root and shoot, and their root/shoot length ratio were calculated. The samples were oven dried to a constant weight at 80^{o}C for dry weight measurements.

Photosynthetic Pigments

Pigments were extracted from fresh *Raphanus sativus* shoot with 100% acetone following the method used by (Fadeel,. 1962,p 130), then measured and calculated according to (Sestak *et al*., 1971, p. 682).

Extraction and Determination of Nitrogen

Total nitrogen was determined in plant powder after the acid digestion with 1 ml

50% H_2SO_4 and 1 ml 30% perchloric acid, using Bertholet reaction (Chaney and Marbach,. 1962, p 130). Soluble nitrogen were extracted from the dried *Raphanus sativus* shoot tissue with 10% trichloroacetic acid (TCA) and the remaining dried residue was acid digested to obtain the insoluble components. Total amount of free amino acids was estimated in the TCA extract as amino-N (Russell, 1944). Multiplying the total organic nitrogen by 6.25 estimated the crude protein (AOAC., 1995).

Statistical analysis

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

Results

Effects on plant growth

In the pot experiment, the growth response of *R. sativus* at different concentrations as affected by *Rhazya* residue is shown in Tables (1-1&2). At 15 days old *R. sativus* shoot length increased with the increase of *Rhazya* residue up to 6 grams concentration compared to control, whereas at 30 days old *R. sativus* shoot length decreased with the increase of *Rhazya* residue compared to control. At age of 15-days, the increase of *Rhazya* residue caused a significant decline in *R. sativus* root length at low concentration, while at age of 30-days, the increase of *Rhazya* residue caused a significant decline in *R. sativus* root length at high concentration. Reduced root/shoot length ratio of *R. sativus* at ages of 15 and 30 days was significant in all *Rhazya* residue concentrations. The highest root/shoot length ratio inhibition at age of 15 days reached 32.07% at residue concentration of 16 grams, while at age of 30 days, the root/shoot length ratio reached 51.40% at residue concentration of 16 grams.

The dry weight of *R. sativus* at ages of 15 and 30 days did not show any positive or negative variation from the control treatment except at 10-12 and 16 grams residue concentration which showed a significant decrease in dry weight of treated plant.

Table 1-1. Effect of different concentrations of *Rhazya stricta* plant residues on seedling growth of 15 days-old *Raphanus sativus* plant

Residue Concentration (g)	Age			
	15 days			
	Shoot Length (cm)	Root Length (cm)	R/S Length Ratio (%)	Dry Weight (g)
0	6.56 ± 0.46^{b}	5.16 ± 0.89^{bc}	79.05 ± 14.94^{bc}	0.023 ± 0.002^{de}
2	6.8 ± 0.69^{b}	4.98 ± 1.7^{bc}	74.91 ± 30.03^{bc}	0.019 ± 0.001^{bc}
4	7.16 ± 0.55^{b}	3.92 ± 1.32^{b}	55.6 ± 20.66^{ab}	0.025 ± 0.002^{e}
6	8.08 ± 0.68^{c}	$5.2\pm0.56b^{c}$	64.25 ± 1.58^{b}	0.022 ± 0.002^{cd}
8	7 ± 0.67^{b}	5.34 ± 0.54^{bc}	76.96 ± 12.15^{bc}	0.023 ± 0.002^{de}
10	6.28 ± 0.47^{ab}	6.26 ± 1.38^{c}	99.58 ± 19.97^{c}	0.022 ± 0.002^{cd}
12	7.16 ± 0.66^{b}	4.02 ± 1.12^{b}	57.6 ± 22.23^{b}	0.017 ± 0.002^{ab}
16	5.66 ± 0.72^{a}	1.82 ± 0.58^{a}	32.07 ± 9.56^{a}	0.014 ± 0.002^{a}

Table 1-2. Effect of different concentrations of *Rhazya stricta* plant residues on seedling growth of 30-days-old *Raphanus sativus* plant.

Residue Concentration (g)	Age			
	30 days			
	Shoot Length (cm)	Root Length (cm)	R/S Length Ratio (%)	Dry Weight (g)
0	6.50±0.72^{cd}	4.16±0.85^{bc}	64±13.72^{ab}	0.052±0.002^{d}
2	5.32±1.51^{abc}	5.20±1.25^{c}	110.8±62.34^{c}	0.056±0.002^{e}
4	6.04±0.71^{bcd}	3.86±1.17^{b}	64.6±22.09^{ab}	0.064±0.002^{f}
6	6.66±0.83^{d}	4.04±0.79^{bc}	60.8±12.02^{ab}	0.054±0.002^{de}
8	6.14±0.63^{bcd}	4.14±0.51^{bc}	67.40±10.31^{ab}	0.053±0.001^{de}
10	4.40±0.22^{cd}	3.48±0.60^{ab}	53.6±8.84^{a}	0.042±0.002^{c}
12	4.36±0.32^{a}	4.06±0.83^{bc}	93.4±22.81^{bc}	0.033±0.002^{b}
16	5.18±1.08^{ab}	2.58±0.69^{a}	51.40±20.11^{a}	0.025±0.003^{a}

Effects on photosynthetic pigments content

Changes in the various photosynthetic pigments in the shoot system of *R. sativus* for 15 and 30 days with different rates of *Rhazya* plant residue are shown in Figure (1) and Appendix Table (1). At age 15 and 30 days, Chlorophyll a doesn't show any significant difference except at 6 and 16 grams residue concentrations which shows significant decrease in chlorophyll a content after 15 days, while at age 30 days, chlorophyll a content decreased by 16 grams residue concentration(Figure 1-a).

Significant reduction in chlorophyll b content of *R. sativus* was detected by the effect of 10 and 12 grams residue after 15 day and at 30 days age, the chlorophyll b content decreased by 10 - 12 and 16 grams residue concentrations (Figure 1-b).

Concerning chlorophyll a/b ratio of *R. sativus* at 15 days showed no constant trend at different concentrations of *Rhazya* residue, while at 30-days age generally increased with increasing the concentrations of *Rhazya* residue (Figure 1-c). At 15 days age carotenoid of *R. sativus* showed a decrease at lower residue concentration while a significant increase was remarked with increasing residue concentration, whereas at 30 days age an increase in carotenoid content was with the increasing residue concentration (Figure 1-d).

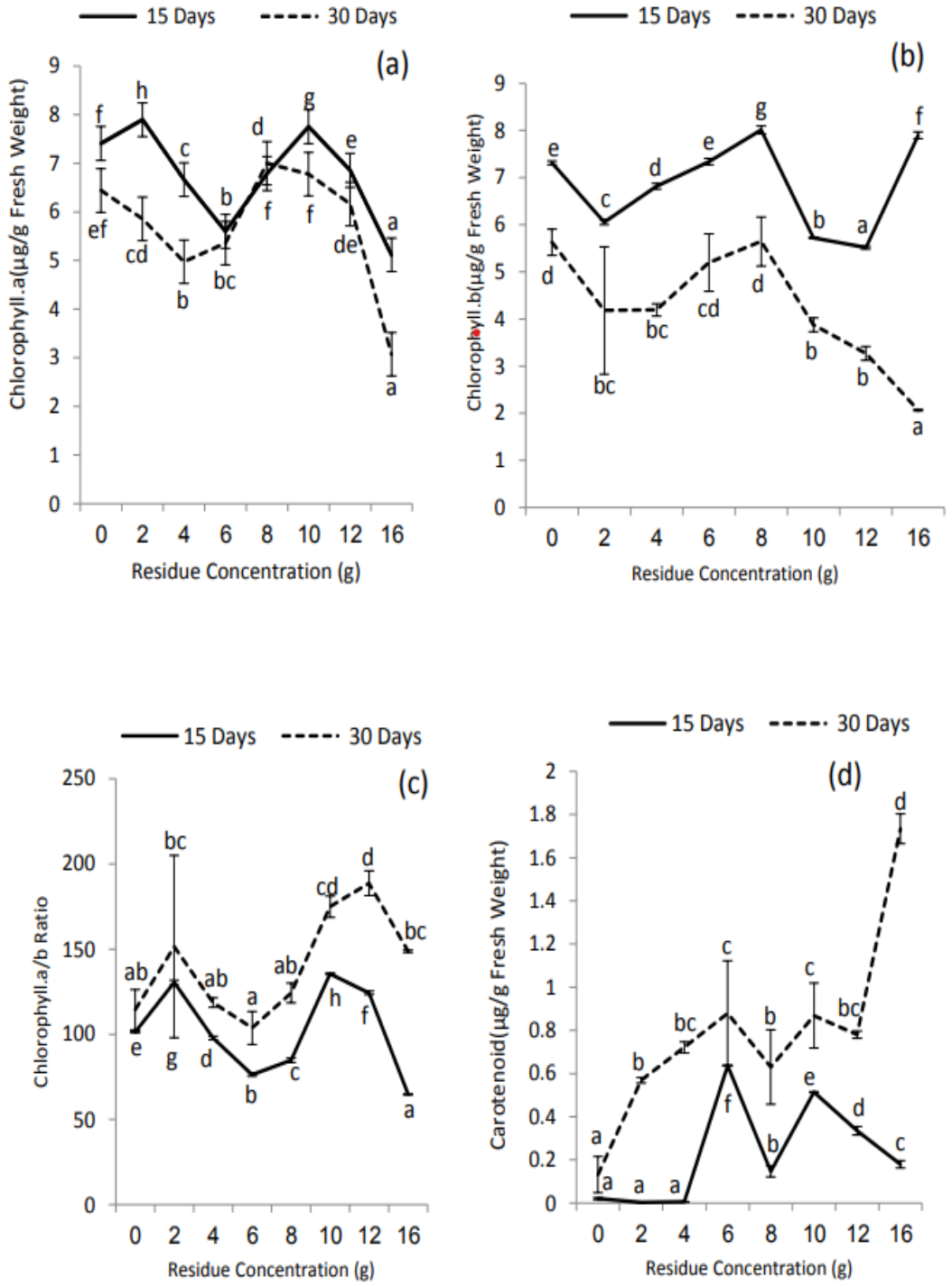
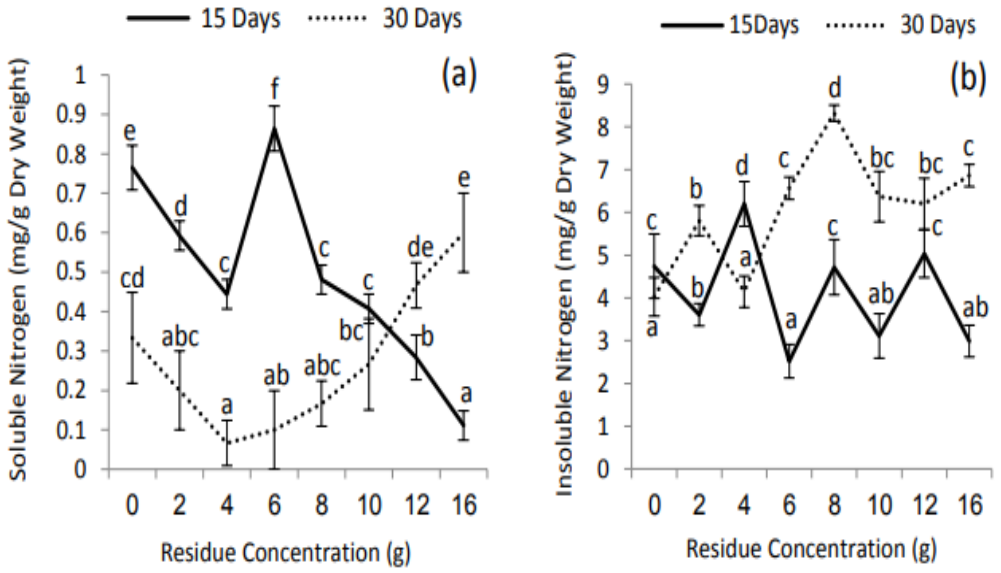


Fig.1. Effect of different concentrations of *Rhazya stricta* plant residues on the photosynthetic pigments (chlorophyll a, chlorophyll b, chlorophyll a/b ratio and carotenoid) of 15 and 30-days-old *Raphanus sativus*. Vertical bars are standard deviation of the mean.

Effects on nitrogen content

The change in the nitrogenous components of 15 and 30-days-old *R. sativus* in response to different *Rhazya* treatments are shown in Figure (2) and Appendix Table (2). At 15-days old, *R. sativus* soluble nitrogen increased by 6 grams, while at higher *Rhazya* residue, the soluble nitrogen of *R. sativus* significantly decreased to (o.11mg/g dry weight) less than the control treatment. In addition after 30-days old, *R. sativus* soluble nitrogen decreased by 4 grams, while at higher *Rhazya* residue, the soluble nitrogen of *R. sativus* significantly increased to (0.60mg/g dry weight) compared with control treatment. (Figure 2-a). With respect to insoluble nitrogen 15 days age *R. sativus* plant did not show any significant difference except at 4 grams residue concentration which showed a marked increase while 6 grams residue showed significant decrease. After 30 days age, plants showed generally increase in insoluble nitrogen with increasing the concentrations of *Rhazya* residue as shown in (Figure 2-b).

In contrast, *R. sativus* at age of 15 days showed generally decrease in free amino acid which is highly marked at 2 grams residue concentration. After 30 days, plants showed a decrease in free amino acid at lower residue concentration while a significant increase was remarked with increasing residue concentration (Figure 2-c). Crude protein of *R. sativus* at 15 days age decreased with the increase of *Rhazya* residue except at 4 grams residue concentration which showed a marked increase in crude protein of *R. sativus*. In addition at 30 days aged plant, crude protein increased with the increase of *Rhazya* residue (Figure 2-d).



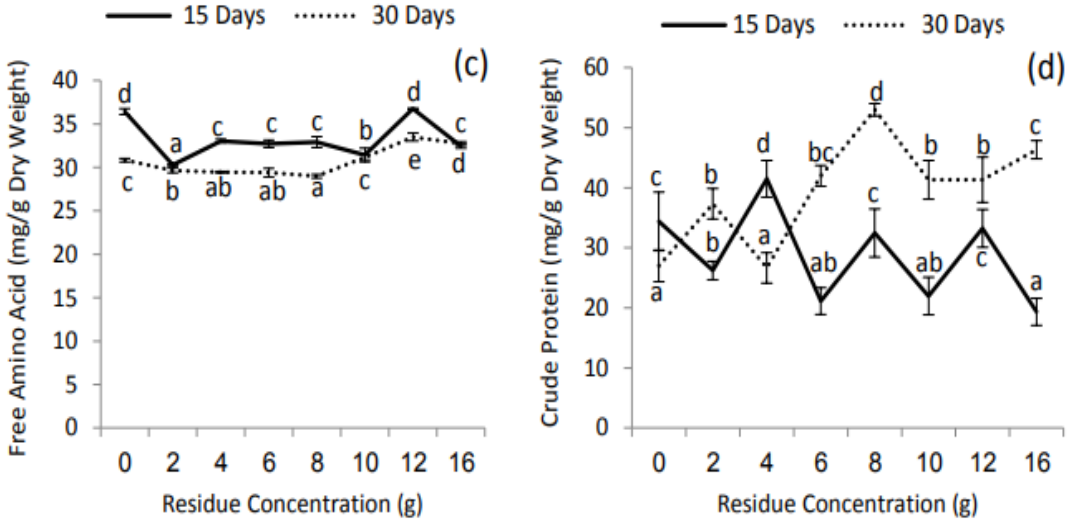


Fig. 2. Effect of different concentrations of *Rhazya stricta* plant residues on the nitrogen fractions (soluble and insoluble nitrogen, free amino acids and crude protein) of 15 and 30-days-old *Raphanus sativus*. Vertical bars are standard deviation of the mean.

Discussion

The results of the present study showed that the residue of *Rhazya stricta* differed in their effects on adult plant growth, photosynthetic pigments and nitrogen content of Radish (*Raphanus sativus*) plant.

Incorporation of *Rhazya* residue into the soil at the low concentrations, the dry weight of *R. sativus* was stimulated particularly at late stages of growth. The positive effect of these treatments indicates that the low quantities of allelochemicals improved *R. sativus* growth. Similarly, Mallik and Watson, (1998) recorded the improvement of soybean and other plants growth by allelochemicals of *Solonium nigram* L. residue. However, the inhibition of root to shoot length ratio was more pronounced at early age stages than at the late stage. Conversely, increasing the rate of *Rhazya* residue caused an inhibition in growth of radish at different ages. These results on the contrary with (Abad., 2019) showed that the inhibition of root to shoot length ratio and dry weight was more pronounced at late age stages than at the early stage. The suppression in *R. sativus* growth parameters with increasing the rates of *Rhazya* plant implies depends on the amount of plant residue in the soil. The accumulation of allelochemicals could have a direct or indirect effect on the *R. sativus* growth by interfering with the action of growth-regulating substances. Similarly, (Al-Wakeel *et al*,. 2007, p 413) who demonstrated the stimulation in root and shoot lengths of 45-day-old pea irrigated with *Acacia nilotica* leaf extract, while the higher concentration were inhibitory.

The residue of *Rhazya* showed both inhibitory and stimulatory effects on photosynthetic pigments of *R. sativus* at different ages. Chlorophylls are the core component of pigment protein complexes embedded in the photosynthetic membranes and play a major role in the photosynthesis. Any changes in chlorophyll content are expected to bring about change in photosynthesis (Reigosa,. 2006, p 315). The

inhibition in Chl a and Chl b were previously reported as a result of allelochemical stress (Singh *et al*,. 2009, p 163) or may be due to the inhibition of chlorophyll biosynthesis or stimulation of chlorophyll degradation or both processes (Yang *et al*,. 2002, p 303). Moreover, (Siddiqui,. 2007, p 306) reported a reduction in chlorophyll content of *Vigna mungo* due to the allelochemicals present in leachates of black pepper which possibly target enzymes responsible for the conversion of porphyrin precursors.

Based on the results, a significant decrease in nitrogen content of *R. sativus* including total amount of free amino acid, soluble and insoluble nitrogen and crude protein was more pronounced at early age stages than at the late stage. This could be due to the higher levels of *Rhazya* allelochemicals, which have harmful effect on nitrogen metabolism (Reigosa,. 2006, p 320). According to the allelopathy definition, it is so evident that allelochemicals could affect all phases of nitrogen cycle involved in plant or microorganisms. When plants take up nitrate, they must use energy to convert it to ammonium form before it can be used (Reigosa,. 2006, p 321). The growth reduction due to missing energy could be an argument for nitrogen reduction in seedlings which treated by allelochemicals, also loosing of nitrogen content in some seedling, may be occurred by limiting or reducing some key factors in nitrogen metabolism such as nitrate reductase and glutamine synthetase (Nie,. 2005). In contrast, the higher concentrations of *Rhazya* residue stimulated the contents of soluble and insoluble nitrogen and crude protein of *R. sativus* in the late stage. This effect could be related to the interaction of *Rhazya* allelochemicals with nitrogen uptake and metabolism. On the contrary with, (Al-Wakeel *et al*,. 2007, p 416) demonstrated that the content of total nitrogen (their insoluble form), increased with lower *Acacia* residues whereas all nitrogen fraction declined by increasing *Acacia* residues.

Conclusion

The present investigation revealed that *Rhazya stricta* residue plant contain some substances which have inhibitory and stimulatory effects on *Raphanus sativus*.

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Appendix Table 1. Effect of different concentrations of *Rhazya stricta* plant residues on the photosynthetic pigments of 15 and 30-days-old *Raphanus sativus* plant.

Residue Concentration (g)	Age							
	15 days				30 days			
	Chlorophyll. a (µg/g Fresh Weight)	Chlorophyll. b (µg/g Fresh Weight)	Chlorophyll. a/b Ratio (%)	Carotenoid (µg/g Fresh Weight)	Chlorophyll. a (µg/g Fresh Weight)	Chlorophyll. b (µg/g Fresh Weight)	Chlorophyll. a/b Ratio (%)	Carotenoid (µg/g Fresh Weight)
0	7.41 ± 0.02^{f}	7.31 ± 0.042^{e}	101.3 ± 0.28^{e}	0.02 ± 0.004^{a}	6.44 ± 0.67^{ef}	5.62 ± 0.27^{d}	114.46 ± 11.76^{ab}	0.13 ± 0.08^{a}
2	7.9 ± 0.02^{h}	6.05 ± 0.055^{c}	130.4 ± 1.42^{g}	0.01 ± 0.001^{a}	5.85 ± 0.29^{cd}	4.18 ± 1.35^{bc}	151.45 ± 53.48^{bc}	0.56 ± 0.01^{b}
4	6.66 ± 0.01^{c}	6.82 ± 0.060^{d}	97.8 ± 1.03^{d}	0.01 ± 0.002^{a}	4.97 ± 0.15^{b}	4.19 ± 0.13^{bc}	118.60 ± 2.70^{ab}	0.72 ± 0.02^{bc}
6	5.6 ± 0.01^{b}	7.34 ± 0.069^{e}	76.3 ± 0.89^{b}	0.64 ± 0.002^{f}	5.35 ± 0.27^{bc}	5.19 ± 0.60^{cd}	103.75 ± 9.74^{a}	0.87 ± 0.24^{c}
8	6.79 ± 0.03^{d}	8.01 ± 0.086^{g}	84.7 ± 1.28^{c}	0.15 ± 0.027^{b}	6.99 ± 0.32^{f}	5.64 ± 0.51^{d}	124.25 ± 5.82^{ab}	0.63 ± 0.17^{b}
10	7.75 ± 0.02^{g}	5.72 ± 0.002^{b}	135.5 ± 0.44^{h}	0.51 ± 0.003^{e}	6.77 ± 0.21^{f}	3.87 ± 0.14^{b}	174.9 ± 6.24^{cd}	0.86 ± 0.15^{c}
12	6.86 ± 0.02^{e}	5.52 ± 0.032^{a}	124.3 ± 1.08^{f}	0.34 ± 0.020^{d}	6.16 ± 0.06^{de}	3.27 ± 0.14^{b}	188.69 ± 7.31^{d}	0.78 ± 0.02^{bc}
16	5.12 ± 0.03^{a}	7.9 ± 0.072^{f}	64.8 ± 0.3^{a}	0.18 ± 0.017^{c}	3.07 ± 0.01^{a}	2.06 ± 0.02^{a}	148.71 ± 0.76^{bc}	1.73 ± 0.07^{d}

Appendix Table 2. Effect of different concentrations of *Rhazya stricta* plant residues on the nitrogen fractions of 15 and 30-days-old *Raphanus sativus* plant.

Residue Concentration (g)	Age							
	15days				30 days			
	Soluble Nitrogen (mg/g Dry Weight)	Insoluble Nitrogen (mg/g Dry Weight)	Free amino acid (mg/g Dry Weight)	Crude Protein (mg/g Dry Weight)	Soluble Nitrogen (mg/gm Dry Weight)	Insoluble Nitrogen (mg/g Dry Weight)	Free amino acid (mg/g Dry Weight)	Crude Protein (mg/g Dry Weight)
0	0.76 ± 0.06^{e}	4.74 ± 0.75^{c}	36.42 ± 0.34^{d}	34.41 ± 4.88^{c}	0.33 ± 0.12^{cd}	4.02 ± 0.44^{a}	30.84 ± 0.19^{c}	27 ± 2.64^{a}
2	0.59 ± 0.04^{d}	3.6 ± 0.26^{b}	30.29 ± 0.09^{a}	26.23 ± 1.54^{b}	0.2 ± 0.1^{abc}	5.80 ± 0.35^{b}	29.65 ± 0.26^{b}	37.33 ± 2.51^{b}
4	0.44 ± 0.04^{c}	6.2 ± 0.53^{d}	33.04 ± 0.2^{c}	41.51 ± 3.06^{d}	0.07 ± 0.06^{a}	4.14 ± 0.37^{a}	29.44 ± 0.10^{ab}	26.66 ± 2.51^{a}
6	0.86 ± 0.06^{f}	2.52 ± 0.39^{a}	32.72 ± 0.43^{c}	21.14 ± 2.25^{ab}	0.1 ± 0.1^{ab}	6.56 ± 0.26^{c}	29.39 ± 0.50^{ab}	42 ± 1.73^{bc}
8	0.48 ± 0.04^{c}	4.72 ± 0.65^{c}	32.9 ± 0.62^{c}	32.48 ± 3.98^{c}	0.17 ± 0.06^{abc}	8.32 ± 0.18^{d}	28.98 ± 0.22^{a}	53 ± 1^{d}
10	0.41 ± 0.04^{c}	3.11 ± 0.52^{ab}	31.43 ± 0.77^{b}	21.99 ± 3.13^{ab}	0.27 ± 0.12^{bc}	6.37 ± 0.58^{bc}	31.17 ± 0.31^{c}	41.33 ± 3.21^{b}
12	0.28 ± 0.06^{b}	5.04 ± 0.56^{c}	36.74 ± 0.07^{d}	33.25 ± 3.16^{c}	0.47 ± 0.06^{de}	6.19 ± 0.59^{bc}	33.49 ± 0.46^{e}	41.33 ± 3.78^{b}
16	0.11 ± 0.04^{a}	2.99 ± 0.37^{ab}	32.47 ± 0.26^{c}	19.36 ± 2.25^{a}	0.6 ± 0.1^{e}	6.86 ± 0.25^{c}	32.77 ± 0.18^{d}	46.33 ± 1.52^{c}

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