Original Research Article

Effect of alkaloids extracted from local lupine plant on growth and yield in pepper

Abstract

Because of the increased demand for pepper due to its nutritional content and high acceptance, the study goal is to improve the growth and productivity of the locally produced pepper crop by using active biological substances such as alkaloids (free of carbohydrates) extracted from bitter lupine.Alkaloids extract was added to each plant at concentrations (0, 500, 100, 1000, 2000) mg d.w./200 ml water and its effecton vegetative evolution, output, and quality of fruit ~~(~~*Capsicum annuum*,~~)~~cultivar Rumi Suez were evaluated. The experiment was conducted under greenhouses in the Agricultural Research Center/Ismailia Station during the winter agricultural seasons 2019/2020 in sandy soil usingthree replications, RCB designand the differences between the means were tested according to Duncan's test at 0.05 probability. The results showed a considerable increment in the vegetative development indicators, including~~(~~plant height, main branches number, leaf area, leaves number, wet and dried weighing of leaves and stems and chlorophyll leaves level, in addition to~~) and~~ productivity ~~(~~yield per plant, total yield~~)~~, vitamin C, and phenols when treated with alkaloids extracted. It was found that the mean number of fruits, yield per plant, total yield, vitamin C content, phenols, and fruit quality increased with increasing concentrations of alkaloids used.

Keywords: Alkaloids; lupine; growth; yield; pepper.

1. Introduction

The sweet pepper plant (*Capsicum annuum. L*) is the third most important crop at family of Solanaceae after tomatoes and potatoes [3]. Its importance comes through its contribution to providing the human body with important energy compounds for building, as every 100 gm of fresh fruits contains about 4.8% carbohydrates and 1.2% protein, in addition to some mineral salts such as potassium, calcium and iron, as well as fluorine, which protects teeth from decay [18]. Sweet pepper is the richest of all vegetables in vitamin C, as one fruit weighing 74 g can meet the necessary requirements of vitamin C for an adult human during one day beside a higher amount of vitamin A, B and other vitamins essential for growth [24]**.**The importance of the pepper crop is due to its high nutritional and health value, as pepper contains a high percentage of vitamin C and vitamin A, which helps treat stomach ulcers. It is characterized by large amounts of mineral such as sulfur, magnesium, iron, calcium, and phosphorous. It is an analgesic for pain, anti-bacterial, control with sugar level in the blood, anti-cancer, reduce the proportion of harmful triglycerides and encouraging fibers degrade in the body [35]**.** **Nishinoet al.** [27]mentioned that pepper fruits have medicinal properties; It is used as a circulatory stimulant, antipyretic, an astringent in cases of diarrhea, an appetizer, and a killer of germs, as well as its role in reducing the risk of cancerous diseases. Pepper fruits contain capsaicin, which important role in the pharmaceutical industry, as it is used in the treatment of arthritis, stomach pain, flatulence, asthma and cough, as well as it will work in the treatment of seasickness and dental pain [25]. The carotenoids in peppers include capsanthin and carotene (zeaxanthin, β-cryptoxanthin, lutein, α- and β-carotene [1]. Carotenoids' cancer-preventive activities connected by antioxidant properties [4].

One of the modern methods to increase agricultural production is using extracts rich in natural plant hormones, vitamins, and some macro and micronutrients for their positive effect in stimulating important physiological and biological activities in the plantthat leads to an increase in plant growth, productivity and enhancing the quality of the yield [19].

Numerous studies indicated that there are some plant extracts encouraging the characteristics of vegetative growth and yield for many plants.Thismay be due to the numerous natural chemical compounds found there, which vary in kind and as well as different species, plant parts,and stages ofplant growth and environmental conditions to which they are exposed [5]. In view of the negative side effects that industrial chemicals may pose to humans, the environment and the living organisms that live in them, the trend has been towards finding alternatives to natural compounds.Products that can have an effect similar to what industrial chemical compounds do [9]**.** [31]stated that the recent global trend "aims to use natural products to preserve the environment and avoid side effects, as biologists have tended to use natural plant extracts as substitutes for industrial chemical compounds [2].

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Lupine consumption has grown in recent years due to the excellent nutritional value of its flour. The flour is higher protein content and excellent nutritional functions. Pastryand bakery products, concentrated protein, and other products like yoghurt, and milk free of lactose are manufactured fromlupine flour [22; 14]. However, it is necessary to remove the existing alkaloids that causing a bitter taste. Alkaloids' presence of lupine in higher doses is toxic and causes respiratory depression, a neuromuscular blockage, cyanosis, cardiac arrest, and cramps [8]. Alkaloids that reach up to 5% of the dry weight of lupine seeds by different technical, which represent around 8-10% of nitrogen contained in lupine seeds [39]. Bitter lupine contained between 1.68 and 2.53% alkaloids [26]. The bitter seed of lupine species had not only a high protein content but also a variety of biological activity substances such as alkaloids, raffinose, phenolic compounds, and others [13]. Lupine alkaloids are a good nitrogen source for seedlings [36]. Bitter lupine extracts improve the development and productivity of a variety of cultivated plants [40]. The beneficial effect of lupine extracts on the growth and yield of various cultivated plants is well known[16; 10; 29]. The alkaloid consist of six important alkaloids: sparteine (19%), 3-hydroxylupanine (12%), hydroxy tetrahydrorhombifoline (5%), ammodendrine (3%), 5,6-dehydrolupanine (2%), 17-oxolupanine (2%), and 19 additional minor alkaloids [37]. Many researches have shown that using lupine extract often boosts yield. [12]shown that lupine extracts improved the development and yield of several planted plants. They discovered that extracts derived from de-bittering farmed species of alkaloid-rich lupine seeds may be used as yield enhancing agents in plant culture. [40] investigated the effect of a extract (after sugar precipitation) from different lupines seeds on lettuce and tomato growth and yield. These authors' findings corroborate and demonstrate that extracts derived from several lupine species have a positive influence on the development and production of diverse plants. The differences in chemical structure of the extract, dosages, plant studied, and other factors may all influence the impact of the extracts. Sugars in the extracts do not appear to have any influence on their biological activity. [29]found that applying the extract contains a low sucrose to soil in different dosages ranged between (80 to 1600 mg dw/pot) enhanced paprika fruit output and vegetative mass especially at higher doses. [17]studied the effect of using bitter lupine extract on the growth and productivity of different crops as a growth regulator (spraying in amounts up to 60 kg/ha) or as a fertilizer (applications up to 250 kg/ha). Spraying with extracts as a growth regulator proved successful as the yield of winter wheat increased by 4%, corn by 8%, soybean by 18% and potato by 23%. While when the extract was used as a fertilizer when applied to the ground, the yield of winter wheat, corn and Chinese cabbage increased by up to 17, 43 and 57%, respectively. As these extracts, increase flowers number, the number of knotted fruits and the productivity of the plant. It is well known that lupine seed extracts are high in amino acids. Using amino acids as a spray on the vegetative structure or adding to soil is improve plant growth and productivity by increasing the tissue protein content by building new types of proteins and enzymes necessary to regulate metabolic activities or activate antioxidants to make the plant more tolerant to the stresses subjected. Treating with amino acids raise the efficiency of the photosynthesis process, thus giving the best vegetative growth, increasing the number of fruits and the total yield, beside to improving the qualitative characteristics of the fruits represented by increasing the percentage of sugars, total solids, total phenols content, and antioxidants[32].

The study aimed to improve the growth and productivity of the local cultivar of pepper by alkaloids extracted from bitter lupine.

2. Materials and methods

2.1. Plant materials and treatments

The experiment achieved in the Agricultural Research Station in Ismailia, Egypt, during the 2019/2020 winter seasons in a greenhouse on sandy soil. The pH of the soil was 6.72, organic matter (1.8 g/kg), EC (1.11dSm^{-1}) at 25°C, total N (0.03 g/kg), and total phosphorus (0.02 g/kg). The cultivar of Romy Suez seedlings was cultivated on 2 January.The experiment was designed with completely randomized plots, five treatments, three replications, fifteen experimental plots, and a 10 m^{2} area for each experimental plot. The spacing between rows were100 cm while they were 50 cm between plants. The dried extract of alkaloids at (0, 100, 500, 1000 and 2000 mg) were mixed by 200 ml water and added to the soil around each plant after two weeks of seedling and then after a month.

2.2. Preparation alkaloid from bitter lupine

The seeds were extracted by ethanol 50% according to [11], then different sugar were precipitated from the extract by ethanol 98% [12], the supernatant liquid was condensed by rotary,anddried by oven under vacuum at temperature no exceeded about 50 ºC.

2.3. Measurements

2.3.1. Vegetative characters

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During the flowering stage, three plants randomlychosen from each plot to evaluate all vegetative measures like plant height, branches number, number of leaves, leaf area, shoot and leaves fresh weight, while shoot and leaves dry weight (after dried at 70 ° C until they reached a consistent weight). The dry weight disc technique established byRhoads and Bloodworth[30]was used to estimate the leaf area. The chlorophyll pigments were taken from the fourth leaf and chlorophyll a, b, and carotenoids determination according to [21].

2.3.2. Plant yield

Plant yield, plot yield, and total yield/fed were all measured during the harvesting time.

2.3.3. Fruit quality included

2.3.3.1. TSS

For these propose, 10 g of samples were pushed through a cheesecloth. TSS of juices was measured at 20°C using an Abbe (C10) refractometer made in the United States.

2.3.3.2. pH

The pH was determined using the Jenway instrument 3510, which was manufactured by Bibby Scientific (UK).

2.3.3.3. Acidity

Acidityand vitamin C were estimated by titration of sodium hydroxide (0.1 M) and the indophenol method according to [15].

2.3.3.4. Total phenolic

Total phenolic compounds were identified using as reported by[28].

2.2.4. Statistical analysis

Data were analyses using SPSS 16 software, and means were compared using Duncan's test with a probability of 5%.

3.Results and Discussions

3.1. Effect of bitter lupine seed alkaloids on plant height, number of branches, leaves and leaf area of the pepper plant.

Table 1.Effect of bitter lupine seed alkaloids on the length, number of branches, number of leaves and leaf area of pepper plant.

	Plant length cm		Branch No.		Leave No.		Leaves area cm^2	
	S1	S2	S1	S2	S1	S2	S1	S2
E_0	43^e	46^e	3.0^e	3.7^d	152^e	171^e	577.3^e	644.1^e
E_{100}	45^d	47^d	3.5^d	3.8^d	158^d	176^d	614.3^d	720.6^d
E_{500}	48^c	50^c	4.0^c	4.2^c	162^c	183^c	703.6^c	842.4^c
E_{1000}	49^b	54^b	4.5^b	4.7^b	167^b	187^b	793.2^b	936.3^b
E_{2000}	52^a	62^a	5.0^a	5.4^a	169^a	193^a	854.6^a	1006.9^a

E_0: no extract (control); E_{100}: addition extract at 100 mg; E_{500}: addition extract at 500 mg; E_{1000}: addition extract at 1000 mg; E_{2000}: addition extract at 2000 mg.

Regarding plant height, it should be mentioned that each plant's length was greatly enhanced compared to the control when alkaloid extract was added at doses ranged between 100 to 2000 mg to each plant. The number of branches, leaves, and leaf area of the pepper plant increased in direct proportion to the dose or concentration of alkaloids applied to each plant, yielding the same results over the course of the two growth seasons. It is evident from the findings that the bitter lupine seed alkaloids promoted length, branching and increased leaf number and area. All of these characteristics are linked by an increase in the number of fruits and producing the necessary nutrients for fruit formation, which increase production and quality.

3.2. Effect of bitter lupine seed alkaloids on dry and fresh weight of stem and leaves of pepper plant

Table 2. Effect of bitter lupine seed alkaloids on dry and fresh weight of stem and leaves of pepper plant.

	Stem fresh weight		Stem dry weight		Leaves fresh weight		Leaves dry weight	
	S1	S2	S1	S2	S1	S2	S1	S2
E_0	28.4^e	30.6^e	3.9^e	4.3^e	24.9^e	26.8^e	4.7^e	5.3^e
E_{100}	32.6^d	32.8^d	4.4^d	5.0^d	27.8^d	29.0^d	5.2^d	6.0^d
E_{500}	34.6^c	38.9^c	4.8^c	5.4^c	30.4^c	34.1^c	5.7^c	6.7^c
E_{1000}	40.1^b	43.5^b	5.6^b	6.1^b	35.3^b	38.1^b	6.7^b	7.5^b
E_{2000}	42.6^a	47.1^a	5.9^a	6.6^a	37.4^a	41.3^a	7.1^a	8.1^a

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E_0: no extract (control); E_{100}: addition extract at 100 mg; E_{500}: addition extract at 500 mg; E_{1000}: addition extract at 1000 mg; E_{2000}: addition extract at 2000 mg.

Raised concentration of the plant's added dosage from the bitter lupine seed alkaloids, which increased the weight of the fresh stem over the two agricultural seasons. Weight gain increased in direct proportion to the amount of alkaloids provided per plant. The addition of alkaloids with a concentration of 2000 mg/plant recorded the highest fresh weight of a stem during the planting seasons (42.6, 47.1 gm) compared (28.4, 30.6 gm) to control. The same results were obtained for the dry stem weight. This comes naturally as a result of increasing plant length, leaves number and leaves area. The results of leaves fresh and dry weight not far from the same path as the results. Both increased directly by increasing the concentration of added extract.Theextract at 2000 mg recorded the highest leaves dry and fresh weight during the two growing seasons, mainly due increasingleaves number and area leaf plant. Alkaloids gave the same enhancement for vegetative mass and dry vegetative mass on paprika with all doses ranging from 80 to 1600 mg/plant [29].

3.3. Effect of bitter lupine seed alkaloids on the pigments present in the leaves of pepper plant.

Table 3. Effect of bitter lupine seed alkaloids on leaves pigments of pepper plant.

	Chlorophyll a ppm		Chlorophyll b ppm		Carotenoids ppm		Chla+chl b	
	S1	S2	S1	S2	S1	S2	S1	S2
E_0	3.60^{e}	3.96^{e}	2.80^{e}	3.08^{e}	1.84^{e}	2.19^{e}	6.45^{e}	7.04^{e}
E_{100}	3.79^{d}	4.02^{d}	2.87^{d}	3.22^{d}	1.92^{d}	2.34^{d}	6.66^{d}	7.24^{d}
E_{500}	3.84^{c}	4.14^{c}	2.94^{c}	3.36^{c}	1.98^{c}	2.46^{c}	6.78^{c}	7.50^{c}
E_{1000}	3.99^{b}	4.27^{b}	3.02^{b}	3.48^{b}	2.13^{d}	2.57^{b}	7.01^{b}	7.75^{b}
E_{2000}	4.12^{a}	4.39^{a}	3.12^{a}	3.62^{a}	2.25^{a}	2.77^{a}	7.24^{a}	8.01^{a}

E_0: no extract (control); E_{100}: addition extract at 100 mg; E_{500}: addition extract at 500 mg; E_{1000}: addition extract at 1000 mg; E_{2000}: addition extract at 2000 mg.

It is worth mentioning that the leaves are considered as the plant's food factory, due to the presence of dyes, particularly chlorophyll, which performs optical building and supplies the plant with food and energy.It is noted that the different concentrations of the extracted alkaloids increased the level of green pigments and carotenoids in the leaves of the pepper plant during the two growing seasons, which is positively reflected on the yield of one plant, fruits number, final fruits weight and quality. Addition extract at 2000 mg for each plant worked to obtain the highest level of chlorophyll a (4.12 and 4.39 ppm), b (3.12 and 3.62 ppm), c (2.25 and 2.77 ppm), a + b (7.24 and 8.01ppm) during the two seasons.

3.4. Effect of bitter lupine seed alkaloids on plant yield, experimental plots and total yield per fad of pepper crop

Table 4. Effect of bitter lupine seed alkaloids on plant yield, experimental plots and total yield per fad of pepper crop.

	Plant yield kg		Plot yield kg		Total yield ton/fed	
	S1	S2	S1	S2	S1	S2
E_0	0.48^{e}	0.49^{e}	10.55^{e}	10.36^{d}	4.01^{e}	4.14^{e}
E_{100}	0.50^{d}	0.52^{d}	11.07^{d}	10.38^{d}	4.23^{d}	4.36^{d}
E_{500}	0.57^{c}	0.57^{c}	12.33^{c}	11.45^{c}	4.76^{c}	4.81^{c}
E_{1000}	0.62^{b}	0.63^{b}	13.38^{b}	12.69^{b}	5.20^{b}	5.33^{b}
E_{2000}	0.66^{a}	0.68^{a}	14.26^{a}	13.64^{a}	5.57^{a}	5.73^{a}

E_0: no extract (control); E_{100}: addition extract at 100 mg; E_{500}: addition extract at 500 mg; E_{1000}: addition extract at 1000 mg; E_{2000}: addition extract at 2000 mg.

For a single plant, pepper production in season 2 (0.68 kg) is higher than the first season (0.66 kg), as well as increased plant production from the pepper crop by increasing the concentration of added extract. The addition of alkaloid extract at 2000 mg per plant resulted in the greatest plant yield throughout the planting seasons (0.66 and 0.68) kg/plant against (0.48 and 0.49) kg/plant for

the control. This rise in single plant yield as a result of increasing extract concentration has been mainly mirrored in the increase plot yield and total yield of the faddan, that increase with higher extract concentration. This reveals the strong influence of bitter lupine alkaloids on enhancing plant yield and faddan yield of peppers grown in the greenhouse under sandy soil conditions.The positive influence of bitter lupine seed alkaloids extracts at plant yield, experimental plots and total yieldin numerous cultivated plants were revealed by [10;6;29].This is may be the effect of alkaloids of lupine extract which operate as photosynthesis catalysts, [38], or their effect as induce nitrate reductase such as cytokinins and dihydrozeatin
[20].

3.5. Effect of bitter lupine seed alkaloids on the fruit properties of pepper plant.

Table 5.Effect of bitter lupine seed alkaloids on the fruit properties of pepper plant.

	Acidity%		**Vitamin C mg/100g on (FW)**		**Total phenols mg/100g on (FW)**	
	S1	S2	S1	S2	S1	S2
$\mathbf{E_0}$	0.66[e]	0.73[e]	265.20[e]	293.70[e]	28.15[e]	36.40[e]
$\mathbf{E_{100}}$	0.60[d]	0.67[d]	276.27[d]	318.30[d]	31.42[d]	39.18[d]
$\mathbf{E_{500}}$	0.55[c]	0.64[c]	288.30[c]	333.50[c]	33.14[c]	44.53[c]
$\mathbf{E_{1000}}$	0.44[b]	0.56[b]	307.70[b]	352.50[b]	38.72[b]	47.73[b]
$\mathbf{E_{2000}}$	0.42[a]	0.48[a]	332.90[a]	364.63[a]	44.62[a]	51.90[a]

E_0: no extract (control); E_{100}: addition extract at 100 mg; E_{500}: addition extract at 500 mg; E_{1000}: addition extract at 1000 mg; E_{2000}: addition extract at 2000 mg.

Fruit acidity reduced through the two planting seasons due to the increased concentration of applied extract. The first season saw a sharper fall than the second.
The range of vitamin C in the fruits improved with the rise in the level of alkaloids added to the plant. The maximum vitamin content was recorded in the second season with a value of 364.63 mg/100 gm on a fresh weight basis by adding 2000 mg of alkaloids to the plant.
Polyphenols are particularly noteworthy among phytochemicals due to their free radical scavenging characteristics and in vivo biological activity. Peppers (*Capsicum* sp.) are known for being rich in bioactive compounds like polyphenols, that associated with reduction the risk of cancer and coronary heart illness [23]. They are ranged between from 20 to 21mg/100 g for the different genotypes of peppers [7]. Phenols are among the most powerful antioxidants present in plants, and their content increased dramatically in the second season of cultivation compared to the first. Its content was alsoenhanced in pepper plant fruits by increasing the concentration of alkaloid extract applied. The additional 2000 mg alkaloids for plant during the second season resulted in the greatest rise, with a value of 51.9 mg/100 g. Fruits' antioxidant activity can be enhanced by phenolic components and ascorbic acid [34]. At full maturity, pepper fruit contains the highest concentration from ascorbic and phenolic compounds [33].

4. Conclusion

The previous research findings confirm the economic importance of reusing and recycling manufacturing waste, like resulting from the use of bitter lupine in manufacturing. It is considered alternative sources, whether for fertilizing or growth regulators, and their importance grows as a result of the fact that they are generated from natural sources instead of industrial sources that work to pollute the environment or plant. The policy of recycling agricultural or food waste to reuse it is seen as a successful policy that has proven effective for a variety of purposes. The residues arising from bitter lupine seeds extract, which is high in alkaloids, showed to be worthy of increasing their usage in agriculture to encourage productivity and quality increases. The results proved greater branch number, plant height, leaves number and area, stem fresh weight, stem dry weight, leaves fresh weight, leaves dry weight, chlorophyll, carotenoids, plant yield, plot yield, total yield, vitamin C, total phenols with increasing the concentration of alkaloids added.

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