Plant Archives Vol. 20, Supplement 2, 2020 pp. 116-125 **e-ISSN:2581-6063 (online), ISSN:0972-5210**

EFFECT OF BIOFERTILIZER AND CHITOSAN ON MEDICINAL ACTIVE COMPOUNDS OF SALTY STRESSFUL VINCA PLANTS

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Abstract

Two factorial experiments carried out in the research center at college of technical with the aim of increasing the concentration of medically active compounds in the leaves of vinca plants. In the first experiment, different concentrations of chitosan were used (0, 20 and 40 mg/l^{-1}) as a spray on the leaves, and the second factor was use and non-use of bacteria *Azospirillium* for stressful plants at several salinity levels (0, 3 and 6 ds) planted in plastic pots. As for the second experiment, the first factor was the use of different amounts of Bokashi (0, 10, 20, 30 and 40%) from the weight of the pot, while it was the second factor use and non-use of bacteria *Azospirillium*. The experiment was designed according to design (C.R.D.) of five replicates for each treatment, each replicate contains five pots. The results showed that the study factors had a significant effect on the studies characteristics, the interaction gave in the first experiment (40 mg/l^{-1} of chitosan+ *Azospirillium* add + 0.0 NaCl) highest levels of alkaloids compounds) Vincristine 314.9, Vinblastine 301.3, Cathranthine 581.7, Vindoline 406.2**)**, while interaction was given (0.0 of chitosan+don't add *Azospirillium* + 0.0 NaCl) lowest results (126.4, 111.2, 203.3 and 176.3) respectively. While in the second experiment the interaction (20% Bokashi + add *Azospirillium*) gave the highest level from vincristine (558.7), the interaction (30% Bokashi + add *Azospirillium*) gave the highest level from vinblastine (388.55) and cathranthine (871.6) and the interaction (40% Bokashi + add *Azospirillium*) given the highest level from vindoline (487.9), while the interaction (don't add Bokashi and don't add *Azospirillium*) it gave the lowest results for all the characteristics.

***Keywords*:** Vinca plant, Chitosan, Bokashi, *Azospirillium*, alkaloids compounds

Introduction

The Vinca plant is L.G. Don *Catharanthus roseus* is one of the medicinally important plants as it contains many alkaline substances that are close to 130 species, the most important of which are the compounds of Vinblastine and Vincristine, which are used in the treatment of cancer diseases, especially leukemia, because they are able to stop the growth and spread of cancer cells (Hai, 2008; Ferreres *et al*., 2011). It is also frequently used in folklore medicine to treat diabetes and some intestinal diseases and in the treatment of some skin diseases (Saliu *et al*., 2018). In light of the steady population growth to the degree that influenced the growth of plants, pollutants increased and a large proportion of the land became saline and became a problem that caused limited areas suitable for cultivation, so it became necessary to find ways to reduce the crisis and exacerbate this problem to reach the results that meet the ambition to reduce its negative impact (Parviz and Satyawati, 2008). So searching for safe factors such as natural compounds or resorting to natural products that possess distinctive properties, including chitosan, which is a cellulosic substance with many uses, is spread in the outer structures of crustaceans such as shrimp and is extracted from them, it is characterized by its non-toxicity and its biological decomposition and has no effects in the living tissues with the ability to transform the cell Plant from a sensitive cell to a cell that is resistant to various environmental stresses, as farmers enrich the use of agricultural pesticides that cause environmental pollution, as it stimulates the kaitenaes enzyme which is one of the defensive enzymes in addition to its work as an anti-oxidant (Hossain and Iqbal, 2014; Iriti and Varoni, 2015). It has also been proven that the bacterium *Azospirillum* plays an important role in the cycle of major elements in addition to its ability to produce growth regulators, so it contributes to reducing the use of mineral fertilizers, which is an economic burden as well as their polluting effect, and has a role in inhibiting the growth of pathogens and increasing plant tolerance to stress situations (Tomer *et al*., 2016). In addition to the bokashi fertilizer, which is a biochemically fermented organic fertilizer that contains a wide spectrum of microorganisms that colonize the roots, enhance growth and availability of nutrients, and increase the biomass in the rhizosphere. It is considered an important alternative to organic fertilizers because of its high efficiency and its application in soils affected by salinity Increases its organic matter and filtrates the salts deep beyond the root areas (Aceves, 2016). The research aims to increase the concentration of alkaline compounds in plant tissues due to the high medical importance of these substances in addition to their high economic value. Therefore, it was necessary to increase their production and reduce the value of their marketing through the use of environmentally friendly factors and natural resources that do not have a harmful impact on the environment and the consumer.

Materials and Methods

Two independent experiments were conducted in the research fields of the Technical College of Al-Musayyib during the period from 7/9/2018 to 7/8/2019 as the first experiment aimed to study the effect of chitosan and bacterial fertilizer *Azospirillum* and their interactions under the influence of different levels of salt stress, while the second experiment was aimed at Study the effect of bokashi and fertilized *Azospirillum* and their interactions on the effective medicinal compounds of the vinca plant.

Victory Pure White (SAKATA company), Danish origin, planted on 20/2/2019, which is seeds of perennial plants specially imported by one of the agricultural companies with white flowers in cork dishes filled with pitmos by 2 seeds/eyes and placed in a dedicated plastic house For the purpose of propagation in anticipation of any

sudden environmental changes that may affect the germination rate. After the seedlings dawned and reached the appropriate size (3-4) real leaves from the seedlings were removed and transferred to plastic pots size 5 kg with a diameter of 23 cm and a height of 30 cm on the date of 25/4/2019 for the first experiment and 4/28/2019 for the second experiment and the soil pressure was taken into account well when transporting the seedlings to prevent Without the movement of the seedlings, it was preceded by sterilization of the potato soils with the fungicide Chinosol, taking into consideration that all service operations were carried out from the irrigation weeding crops that were applied during the period of the two trials (Gedo, 2015).

Bio-enrichment Preparation

Isolation of the bacterium *Azospirillum* spp. Isolated previously in the graduate laboratory of the University of Kufa-College of Agriculture, then the isolation was developed and multiplied in the laboratories of the Department of Biotechnology Department of the Technical College Musayyib, adding 10 ml of the bacterial farm to 90 ml of distilled water and placed in glass jars of 250 ml capacity mixed well then Serial scares were performed under sterile conditions until 5-10 dilution by transferring 1 mL of the cultured bacterial culture dilution as a biological fertilizer to test tubes containing 9 g of Nutrient Broth (Nfb) liquid culture medium after which the tubes were incubated at 28 °C for a period of 3 days St through the emergence of cyclic growth (pellicle) white color after 1 - 4 mm below the surface after 24-48 hours, which is an indication of a positive result of the growth of bacteria *Azosperillium* spp. (Fadel, 2019). Then a bacterial vaccine (using a sterile needle) was taken from the tubes that gave an indication of growth and then was spread on the surface of a petri dish containing the activated culture medium by transferring 50 ml from the medium in a 250 ml conical flask and incubated the jugs in the incubator at a temperature of 28 ºC for 3 days As the equivalent of 5000 ml of bacterial trap was prepared and developed, which is the sufficient amount that is required to be treated by adding it to the pots soil for both studies, by preparing conical flasks with a capacity of 250 ml each containing 1 ml of the prepared liquid farm by using sterile pipettes. The jugs were incubated in the incubator for a period of time. 2 - 3 days at a temperature 28º m. Prepared bacteria were used as a biological fertilizer, as 20 ml of pure liquid bacterial culture were injected into each pot. For both experiments, a light irrigation procedure was added immediately after the addition of the bacterial farm, then left for 5 consecutive days to ensure its homogeneity with the soil mixture in the cultivation pot and its multiplication.

Prepare the chitosan concentrations

The natural chitosan extracted from Malaysian-made shrimp shells was used in the form of a fine white powder produced by ZOOS Victoria. Dissolve 20 g and 40 g of chitosan in 50 ml acetic acid and complete the volume to 1000 ml distilled water for the purpose of preparing both concentrations used in the research experiment (Abbas, 2016). Spray both concentrations used in the experiment on the leaves until full wetness with a hand sprinkler at the rate of the first two spraying dates were on 20/5/2019 and the second spray was ten days after the first spray.

Preparing the bokashi mixture

The bokashi mixture was prepared at the Organic Fertilizers Preparation Center of the Babylonian Agricultural Directorate for the period from 07/09/2018 to 28/03/2019 with four consecutive steps, namely (collecting the components of the mixture and then the mixing process first and adding the biological fertilizer secondly followed by the fermentation process third and then the incubation process fourth In the beginning, the mixture components, which included wheat bran, rice husks and sheep residues, were mixed in a weight ratio (1: 1: 1). The mixture until reaching the ratio Pric 30-40% as it became the mixture in a doughy. Then the mixture formed in black polyethylene bags was filled with tight closure, and the bags were placed in a dark place for the purpose of increasing the speed of decomposition for about six months, taking into account the continuous stirring of the mixture every 10 days, and then popped in the last stage, which is the incubation stage in a tight 80 kg plastic barrel Closure (Wijayanto *et al*., 2016). It was then placed inside a closed roof away from direct sunlight and covered with a transparent polyethylene bag to prevent air contact with the components of the mixture, with the procedure of opening the barrel cover every two weeks to leak the gas formed inside the barrel and at the end of the incubation stage that lasted for about 45 indicated the presence of white growths on the surface of the mixture And the unpleasant smell disappeared that the mixture was ready for use (Al-Jarrah, 2011). Before using and adding to the soil of the pot, the mixture was washed daily with distilled water and for eight days in a row to get rid of harmful salt compounds.

Transactions and experimental design

Two experiments were carried out (3x2x3) and (5 x 2) according to the design of fully randomized C.R.D. With five iterations per treatment, each repeater contained five pots, one plant per pot. The first factor in the first experiment represented the use of two levels of the bacterial vaccine *Azospirillum* (adding and not adding), while the second factor was spraying three concentrations of the chitosan compound (spraying with distilled water only, 20 and 40 $mg.L^{-1}$), while the third factor represented three levels of saline NaCl is (metering treatment without brine addition, 3 and 6 dysmen). As for the second experiment, the first factor represented adding five levels of bocache (not adding the mixture, 10%, 20%, 30% and 40% of the weight of the soil of the pot) as weight ratios mixed with the river mixture in the pot, while the second factor represented using two levels of the bacterial vaccine *Azosperillium* spp. (Add the vaccine and not add it). The data were analyzed according to GenStat 2008 and the averages were compared according to the least significant L.S.D. difference. The probability level is 0.05% (Asadi, 2019).

Determination of leaf content of medically active compounds using a High Performance Liquid Chromatography (HPLC) device

The leaf samples were taken from 5 replicates after being dried at room temperature until the weight was stable. Then the paper samples were ground and put in the refrigerator until analysis. Prepare the sample solution by taking 0.25 g as it was crushed and ground using a pestle mortar and converted into a fine powder and then dissolved in 10 ml methanol alkaloid using KOH on a water bath with

automatic shaking by Shaker for 3 hours. Then the solution was removed from the water bath and left until complete dryness then the sample was dissolved in 1 ml of methanol and then the sample was filtered with a filter paper and again filtered by a 0.2-micrometer PTFE needle then the filter was then taken for measurement in the HPLC by liquid chromatography process or color separation (Chromatography) using standard solutions of alkaline compounds: Vincristine, Vinblastine Vindoline, Cathranthine (El Amrani, 2015).

Machine Conditions

Alkaline compounds were estimated in the laboratories of the Material Analysis Department of the Ministry of Science and Technology using the modern model HP LC-10AV Liquid Chromatography Shimadzu equipped with a dual injection pump (LC-10V) to determine the retention time and the sample package area for each of the standard solutions for active substances. And the sample solution, when using the Acclaim 120 C-18 separation column with dimensions (150 × 2.1mm ID) and the size of the syringe is 5 micromol and the mobile phase was pushed, which is a solution consisting of (25 micromol Ammonium acetate) with a pH of 8.2 V ratio/V 45/40/15 flow rate of 1.2 ml / min^{-1} and then readings were measured at a wavelength of 297 nm at a temperature of 25 M. The compounds present in the models were measured by comparing the area of the unknown bundles of the model with the known beam areas of the required standard material. This process was repeated on all samples diagnosed under the same separation conditions and the vehicle concentrations were calculated by the following equation (Siddiqui *et al.,* 2011).

$$\text{Concentration of the sample by sample (ppm)} = \frac{(\text{Prototype package space x standard prototype concentration})}{(\text{standard prototype sample space})} \times \text{number of dilutions}$$

Seq	Subject	Retention time minute	area
1	Vindoline	2.36	120562
2	Vincristine	3.61	153698
3	Cathranthine	4.45	112076
4	Vinblastine	5.51	128537

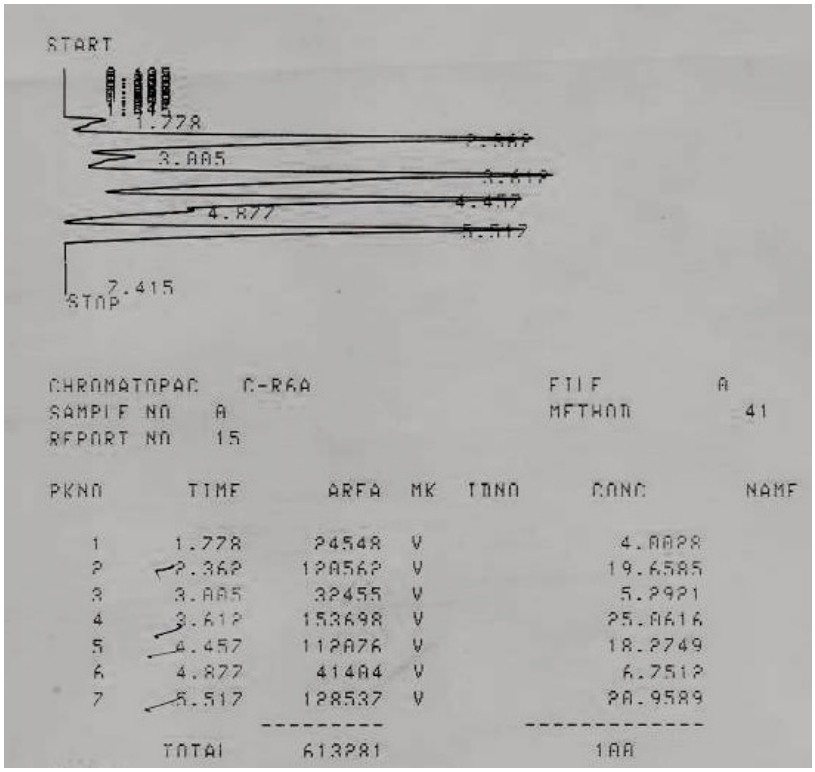

Fig. 1 : Standard Model Curve, Retention Time, and Beam Area for Medicinally Effective Compounds Measured in Leaf Extract for vinca Plants Using HPLC

Results and Discussion

The effect of bacterial and chitosan fertilizer on the medically effective compounds of salty stressful vinca leaves:

(i) Vincristine alkaloid (Vrc) (ppm)

Table (2) data shows that the bacterial fertilizer *Azosperillium* had a significant effect on the leaf content of Vrc alkaloid, as the addition treatment was significantly superior by registering the highest rate of 229.2 ppm compared to 188.3 ppm for the non-additive treatment. The results of the same table also indicated that the chitosan compound had a significant effect on the above characteristic, so spray treatment excelled at a concentration of 40 mg. Liters-1 significantly over the rest of the transactions, as it recorded the highest rate of 247.0 ppm compared to the comparison treatment that recorded the lowest rate of 161.1 ppm. Concerning the effect of saline

tension, the results showed in the same table that there were significant differences in the effect of the saline level of the culture medium, and this effect was inversely related to the increase in the saline tensile strength, as the alkaloid concentration decreased with increasing salinity. The lowest alkaloid concentration was 197.1 ppm.

The overlap of bacterial fertilizer and chitosan had a significant response in the Vrc concentration, as the interference of the fertilizer addition with the concentration exceeded 40 mg. Liter^{-1} of chitosan was significant over all treatments, achieving the highest rate of 266.5 ppm, while the overlap of not adding fertilizer and not spraying with chitosan gave the lowest rate of 152.6 ppm. As for the interference of the enrichment addition with the salt level, the results of the same table showed the superiority of the enrichment addition of + 0.0 mm NaCl, the highest rate of 260.4 ppm, thus outperforming all other factors, while the interference of not adding the bacterial fertilizer with the highest level of NaCl salt has the lowest rate of 183.1 ppm. The results of the same table showed that there were significant differences in the content of the leaves of vinca plants from the Vrc alkaloid caused by the interference of chitosan spray with different levels of salt tension, so the interference treatment achieved 40 mg. As for the treatment of interference, no chitosan spray + 0.0 mm NaCl gave the lowest rate of 145.6 ppm. As for the overlap of the three study factors, it achieved a significant (within the addition of bacterial fertilizer + 40 mg. Liters^{-1} of chitosan + 0.0 mm NaCl) significantly superior to all interference factors except for interference (addition of bacterial fertilizer + 20 mg. Liters^{-1} of chitosan + 0.0 mm. NaCl), as there were no significant differences between them, they recorded the highest rate (314.9 and 302.4 ppm), respectively, while the lowest rate was achieved when overlapping the non-addition of bacterial fertilizer + 0.0 mg. Liters^{-1} of chitosan + 0.0 mm NaCl and reached 126.4 ppm. The reason for excelling may be due to the positive effect caused by the overlap of the three factors between them.

Table 2 : Effect of bacterial and chitosan fertilizer on Vincristine leafy leaf content at several NaCl concentrations

Azosper.* × *Chitosan	**Mlimoz Levels *Salt***			***Chitosan* mg.l^{-1}**	***Azosper.***
	6.0	**3.0**	**00**		
152.6	167.0	164.5	126.4	00	–
184.7	181.2	185.4	187.6	20	
227.5	201.0	214.8	266.6	40	
169.5	174.5	170.1	163.9	00	+
251.6	240.0	212.5	302.4	20	
266.5	218.8	265.9	314.9	40	
11.27	19.52			L.S.D. 0.05	
Average *Azosper.*	*Azosper.* × *Salt*				
188.3	183.1	188.2	193.5	–	
229.2	211.1	216.2	260.4	+	
6.51	11.27			L.S.D. 0.05	
Average *Chitosan*	*Chitosan* × *Salt*				
161.1	170.7	167.3	145.2	00	
218.2	210.6	198.9	245.0	20	
247.0	209.9	240.3	290.7	40	
7.97	13.80			L.S.D. 0.05	
	197.1	202.2	227.0	Average *Salt*	
	7.97			L.S.D. 0.05	

(ii) Vinblastine Alkaloid (Vib)

The results of Table (3) show that there were significant differences caused by the addition of bacterial fertilizer to the culture medium, as the addition of fertilizer treatment was significant by registering the highest rate of 243.6 ppm, while the non-addition treatment recorded the lowest rate of 221.0ppm. The same table data also indicated that the chitosan concentrations had a significant effect on the content of vinca leafs from Vib alkaloid, so spray treatment was recorded at a concentration of 40 mg. Liters^{-1} was significantly superior to other treatments in the experiment, recording the highest rate of 276.1 ppm, while the treatment of distilled water recorded the lowest rate for this The capacity is 170.6ppm. The results of the same table showed that the effect of NaCl salt added to the culture medium had a significant effect on the mentioned trait. The salinity level treatment of 3 mm achieved the highest rate of 239.8 ppm without significantly different from the higher level treatment that recorded a rate of 237.6 ppm while the comparison treatment gave the lowest rate of Vib alkali and its capacity 219.4 ppm. The results of the table showed that the bilateral interaction between the bacterial fertilizer and chitosan had a significant effect in the above characteristic, as the interference of the fertilizer addition with a concentration of 40 mg. Liters^{-1} achieved a significant superiority over all the interference factors, achieving the highest rate of 289.8 ppm, while the comparison treatment achieved the lowest rate of 164.4 ppm Without significant difference with the treatment of enrichment supplementation with spraying with distilled water, which recorded a rate of 176.8 ppm. The interference between the bacterial fertilizer and the salinity levels also had a significant effect on the leaf content of the alkaloid Vib. The interference of the fertilizer addition with the salinity levels 3 and 6 mm was recorded as a significant response in this capacity, achieving the highest rate (249.4 and 248.9 ppm), respectively, without significantly differing between them. While the non additive interference, without the presence of saline tension, recorded the lowest rate of alkali vib of 206.4ppm. The results of the same table also showed that there was a significant effect on the vib alkaloid characteristic due to the interference of chitosan spray with

the salt levels added to the agricultural medium, so the spray overlap was achieved at a concentration of 40 mg. Liters -1 + 0.0 mm. The highest rate for this trait was 283.2 ppm, thus outperforming all other treatments except Interference factors (spraying at a concentration of 40 mg. Liters -1 + 3 and 6 mmol NaCl), while the non-chitosan spray interference + 0.0 mg NaCl scored the lowest rate for this trait of 124.1 ppm. The results of the same table also showed that the triple interference had a significant effect in the Vib alkaloid, so the interference of the enrichment and spraying with a concentration of 40 mg. Liters -1 without NaCl achieved a significant difference in this characteristic over all the characteristics except for the two coefficients (adding fertilizer and spraying with a concentration of 40 mg. Liters -1 + 3 and 6 mm NaCl) at rates of (301.3, 278.9 and 289.3 ppm) respectively, while the lowest rate for this trait was achieved when comparing with a rate of 111.2ppm. The reason for the superiority can be attributed to the positive effect of the three study factors.

Table 3 : Effect of bacterial and chitosan fertilizer on Vincristine leaf leaf (ppm) at several NaCl concentrations

Azosper.* × *Chitosan	**Mlimoz Levels *Salt***			***Chitosan* mg.l^{-1}**	***Azosper.***
	6.0	**3.0**	**00**		
164.4	186.0	195.8	111.2	00	–
236.1	231.9	233.5	242.9	20	
262.4	260.7	261.4	265.0	40	
176.8	197.3	196.1	137.0	00	+
264.1	260.2	273.2	258.9	20	
289.8	289.3	278.9	301.3	40	
13.54	23.46			L.S.D. 0.05	
Average *Azosper.*	*Azosper.* × *Salt*				
221.0	226.2	230.3	206.4	–	
243.6	248.9	249.4	232.4	+	
7.82	13.54			L.S.D. 0.05	
Average *Chitosan*	*Chitosan* × *Salt*				
170.6	191.7	195.9	124.1	00	
250.1	246.0	253.4	250.9	20	
276.1	275.0	270.2	283.2	40	
9.58	16.59			L.S.D. 0.05	
	237.6	239.8	219.4	Average *Salt*	
	9.58			L.S.D. 0.05	

(iii) Vindoline (Vin) alkaloid

Statistical analysis data showed in Table (4) that there is a significant increase in Vin alkaloid due to the addition of bacterial fertilizer to the culture medium, as the addition treatment achieved a significant superiority over the non-addition treatment by achieving the highest rate of 291.2 ppm compared to 236.1 ppm for the non-addition treatment. As for the effect of spraying with chitosan, it affected significantly according to the results shown in the same table. The treatment of spraying with a concentration of 40 mg was recorded. Liter -1 significant difference in the above characteristic with a rate of 310.9 ppm, while the comparison treatment recorded the lowest rate of 212.2ppm. The salinity levels of NaCl also had a significant effect on the above characteristic. The comparison treatment recorded the highest rate of 305.5 ppm to outperform other salt treatments. As for the salinity level treatment 3, it recorded the lowest rate of 240.6 ppm without differing from the level 6 tied treatment that recorded a rate of 244.9 ppm. The same table data also indicated the occurrence of a significant difference in Vin alkaloid as the interference of the enriched addition to the spray was recorded at a concentration of 40 mg. L-1 of chitosan significantly superior to all the interference factors, as it recorded the highest rate of 338.7 ppm, while the comparison treatment recorded the lowest rate for this characteristic of 188.1 ppm. The same table data also showed a significant increase in Vin alkaloid caused by the interference of bacterial enrichment addition with NaCl levels, so the interference of the enriched addition with 0.0 mm NaCl achieved a significant superiority over all other treatments with the highest rate of 345.3ppm, while the lowest rate for this trait was recorded when the interference did not add fertilizer With a salt level 3 mm the lowest rate of 213.8ppm. As for chitosan interference with saline levels, the results of the same table showed significant differences between the treatments, so the spray interference treatment was recorded at a concentration of 40 mg. Liters -1 + 0.0 mm NaCl, the highest rate reached 387.7ppm, to outperform all other interventions, while the lowest rate for Vin alkaloid was recorded. The comparison treatment of 207.6 ppm. The triple interference had a significant effect in this trait, so the interference of the fertilizer and spray addition at a concentration of 40 mg. Liters of chitosan + 0.0 mm NaCl significantly increased all other treatments, recording the highest rate of 406.2 ppm, while the comparison treatment recorded the lowest rate of 176.3ppm. The reason for excelling may be due to the positive effect of the three study factors.

(iv) Cathranthine alkaloid (Cath)

The results of Table (5) showed that the bacterial fertilizer *Azosperillium* had a significant effect on the content of the vinca leaves from Cath Alkaloid. The treatment of enriched fertilizer recorded a significant superiority by registering the highest rate of 444.6 ppm, while the non-addition treatment recorded the lowest rate of 371.2ppm. As for spraying with chitosan, it resulted in a significant superiority according to the data of the same table, and the increase was steady with an increase in the concentration used according to the experiment conditions. The treatment

of spraying with a concentration of 40 mg was recorded. ppm. The levels of NaCl salt had a significant effect, as the level of 6-mmose treatment achieved the highest rate of 425.1 ppm, outperforming all other treatments, while the comparison treatment recorded the lowest rate of this trait of 382.8ppm.

As for the bilateral interactions between the experiment factors, the same table data indicated that the addition of enriched with chitosan had a significant effect in Cath alkaloid, so the treatment of adding fertilizer with spraying at a concentration of 40 mg. Liters -1 achieved a significant superiority over all other treatments by registering the highest rate of 529.0ppm, while The lowest average comparison treatment was 276.0ppm. The addition of the bacterial enrichment addition to the NaCl salt levels resulted in a significant response in the described trait. The addition of the enriched addition with the salt level recorded 451.3 ppm, the highest rate of Cath alkali reached 451.3ppm, thus outperforming all other treatments except for the two enrichment additions without NaCl and the level 3 is noticeable, while it scored The lowest rate for this quality when comparing treatment was 324.1 ppm. The results of the same table showed that the interference between chitosan and NaCl levels had a significant effect on the described trait, as spray interference recorded at a concentration of 40 mg. Liters -1 without the presence of NaCl the highest rate reached 526.0 ppm, thus outperforming all other factors used in the experiment in a gene with the lowest rate for this The adjective when comparing the transaction was 241.4 ppm.

With regard to triple interference, the results of the table showed that there were significant differences due to the interaction of the three factors, the most recent interference of adding fertilizer and sprinkling with a concentration of 40 mg. Liters -1 + 0.0 mm NaCl significant superiority over all other triple interaction factors, giving the highest rate of 581.7ppm, while the lowest rate was recorded For Cath alkaloids when comparing treatment was 203.3ppm. It can explain the reason for the superiority to the positive role brought about by the overlap of the three factors combined in the study.

Table 4 : Effect of bacterial fertilizer and chitosan on Vindoline leaf leaves of Vindoline (ppm) at several NaCl concentrations

Azosper.* × *Chitosan	**Mlimoz Levels *Salt***			***Chitosan* mg.l^{-1}**	***Azosper.***
	6.0	**3.0**	**00**		
188.1	203.1	184.8	176.3	00	–
237.2	243.5	216.7	251.5	20	
283.1	240.3	239.9	369.2	40	
236.3	238.6	231.5	238.9	00	+
298.6	248.8	256.1	390.8	20	
338.7	295.0	314.8	406.2	40	
7.98	13.81			L.S.D. 0.05	
Average *Azosper.*	*Azosper.* × *Salt*				
236.1	229.0	213.8	265.7	–	
291.2	260.8	267.5	345.3	+	
4.60	7.98			L.S.D. 0.05	
Average *Chitosan*	*Chitosan* × *Salt*				
212.2	220.9	208.2	207.6	00	
267.9	246.1	236.4	321.2	20	
310.9	276.7	277.3	387.7	40	
5.64	9.77			L.S.D. 0.05	
	244.9	240.6	305.5	Average *Salt*	
	5.64			L.S.D. 0.05	

Table 5 : Effect of bacterial and chitosan fertilizer on the content of the leaves of the vinca plant from Cathranthine (ppm) at several NaCl concentrations

Azosper.* × *Chitosan	**Mlimoz Levels *Salt***			***Chitosan* mg.l^{-1}**	***Azosper.***
	6.0	**3.0**	**00**		
276.0	328.4	296.2	203.3	00	–
360.3	387.0	395.2	298.7	20	
477.4	481.4	480.7	470.2	40	
330.5	360.9	351.3	279.3	00	+
474.3	478.3	481.1	463.3	20	
529.0	514.7	490.6	581.7	40	
11.56	20.02			L.S.D. 0.05	
Average *Azosper.*	*Azosper.* × *Salt*				
371.2	399.0	390.7	324.1	–	
444.6	451.3	441.0	441.5	+	
6.67	11.56			L.S.D. 0.05	
Average *Chitosan*	*Chitosan* × *Salt*				
303.3	344.7	323.7	241.4	00	
417.3	432.7	438.1	381.0	20	
503.2	498.1	485.7	526.0	40	
8.17	14.16			L.S.D. 0.05	
	425.1	415.8	382.8	Average *Salt*	
	8.17			L.S.D. 0.05	

The effect of bacterial and bukashi fertilizer on the medically effective compounds of vinca leaf

(i) Vincristine alkaloid (Vrc)

The results presented in Table (6) show that there were significant differences in the content of leaves of Alonka plants from the alkali Vrc, as the addition of the enriched bacterial showed a significant superiority in this trait by registering the highest rate of 393.02 ppm, while the non-addition treatment recorded the lowest rate of 273.63 ppm. As for the effect of added bokashi mixed with the medium of cultivation, the level made 20% of the amount of bokashi added to the cultivation bases, a significant difference in Vrc alkaloid by achieving the highest rate of 408.6 ppm, thus outperforming all other transactions except for the level treatment 30% as they did not have significant differences between them. The lowest rate was achieved when comparing the transaction and reached 197.1 ppm. The results of the same table showed that the overlap of both study factors had a significant effect in this capacity, as the interference treatment recorded the addition of the bacterial fertilizer with the level of 20% of the Bukashi highest rate of 558.72 ppm, thus outperforming all other transactions, while the lowest rate was recorded in the comparison treatment and reached 155.89 ppm. Perhaps the reason for the significant increase is explained by the positive contribution of both study factors to the studied characteristic.

Table 6 : Effect of bacterial and bocache fertilizer and their interactions on leaf content of Vinca plant wunca

Average *Azosper.*	***Bokashi*(%)**					***Azosper.***
	40	**30**	**20**	**10**	**0.0**	
273.63	392.14	397.19	258.40	164.51	155.89	–
393.02	371.70	399.35	558.72	396.99	238.33	+
	381.9	398.3	408.6	280.3	197.1	Average *Bokashi*
	interferance	*Bokashi*		*Azosperillium* spp.		L.S.D.
	25.96	18.35		11.61		0.05

(ii) Vinblastine alkaloid (Vib)

The results shown in Table (7) show a significant difference in the content of leaves of Avinca plants from the alkaloid Vib, as the addition of the bacterial fertilizer caused a significant effect by achieving the highest rate of 350.67ppm, while the non-addition treatment recorded the lowest rate of 290.56 ppm. The Bukashi had a significant effect in this capacity, as the level 40% of the amount of Bukashi added to the cultivar pots made a significant difference in the Vrc alkaloid by giving it the highest rate of 373.74 ppm, thus outperforming the comparison and treatment level 10% only without having significant differences with other transactions. The lowest rate was recorded at the comparison transaction, at 280.45 ppm.

The same table also shows that the overlap of the two study factors had a significant effect on the Vib alkaloid, as the interference of the addition of the bacterial fertilizer with the level of 30% of Bukashi recorded a significant superiority over the comparison and treatment coefficients (not adding the fertilizer and the level of 10% of Bukashi) only without having significant differences with The rest of the transactions recorded the highest rate of 388.55 ppm. While the comparison treatment recorded the lowest rate of 258.13 ppm. The reason for the superiority may be explained by the positive effect of the overlap of both study factors.

Table 7 : Effect of bacterial and bocache fertilizer and their interactions on leaf content of vinca plants from Vincristine (ppm)

Average Azosper.	**Bokashi(%)**					***Azosper.***
	40	**30**	**20**	**10**	**0.0**	
290.56	367.70	281.91	280.09	264.97	258.13	–
350.67	379.78	388.55	369.95	312.29	302.77	+
	373.74	335.23	325.02	288.63	280.45	Average Bokashi
	interferance	*Bokashi*		*Azosperillium spp.*		*L.S.D.*
	115.9	81.9		51.8		*0.05*

(iii) Vindoline alkaloid (Vin)

The results of Table (8) indicate the presence of significant differences in the content of the leaves of the vinca plants from the alkaloid Vin caused by the addition of the bacterial fertilizer to the culture medium, as the addition treatment recorded a significant superiority over the non-addition treatment by achieving the highest rate of 347.91 ppm compared to 250.28 ppm for the non-addition treatment. Also, the addition of different quantities of organic-bio-fertilizer to the cultivar bases had a significant effect in Vin alkaloid, as the level treatment of 30% was superior to all other treatments except for the level of 40% by registering the highest rate for this trait, which reached (389.38 and 384.43 ppm) respectively. While the comparison treatment recorded the lowest rate for this trait of 180.0ppm. As for the interference of both study factors, the addition of the bacterial enrichment with the level 40% of the buchachi caused a significant superiority over all other interference factors except for the interference treatment, adding the bacterial fertilizer with the level of 30%, which recorded the highest rate (487.92 and 462.3 ppm), respectively, while The comparison treatment recorded the lowest rate for this trait that was 171.34 ppm without significantly difference with the two coefficients of addition of enrichment without the addition of bocache and the 10% level, which recorded a rate of (188.67 and 211.75 ppm) respectively. This may be due to the positive role of both the bacterial fertilizer and the Bukashi fertilizer in causing the moral effect.

Table 8 : Effect of bacterial and bocache fertilizer and their interactions on the leaf content of vinca plants from Vindoline (ppm)

Average Azosper.	*Bokashi*(%)					*Azosper.*
	40	**30**	**20**	**10**	**0.0**	
250.28	280.94	316.46	252.32	230.35	171.34	–
347.91	487.92	462.30	388.93	211.75	188.67	+
	384.43	389.38	320.63	221.05	180.0	Average *Bokashi*
	interferance	*Bokashi*		*Azosperillium* spp.		L.S.D.
	45.91	32.46		20.53		0.05

(iv) Cathranthine alkaloid (Cath)

The results of Table (9) show that there are significant differences in the content of the leaves of the vinca plant from the alkaloid Cath, the most recent of which was the addition of bacterial fertilizer to the culture medium, as the addition treatment gave the highest rate of 709.68 ppm, thus significantly exceeding the non-addition treatment that recorded the lowest rate of 252.25 ppm. The addition of different quantities of bokashi to the cultivar ponds had a significant effect in Vin alkaloid, as the treatment of the two levels exceeded 30 and 40% significantly over other transactions, registering the highest rate (588.72 and 591.75 ppm), respectively, while the comparison treatment achieved the lowest rate for this trait and reached 241.66 ppm. The results of the same table also showed that the interference of the two study factors had a significant effect on the content of the Wonka leaves from Cath Alkaloid, as the interference treatment exceeded the addition of the bacterial fertilizer with the level of 30% of the buchashi over all the coefficients of the interference except for the overlap of the addition of the bacterial fertilizer with the level of 40% of the bukashi. Significant differences occurred between them, the highest rate for this trait was (871.6 and 851.88 ppm), respectively, while the comparison treatment recorded the lowest rate for this trait of 176.74 ppm without differing significantly with the overlap of not adding fertilizer with the level of 10% of the Bukashi that recorded a rate of 203.94 ppm. The reason for excellence may explain the positive role of both study factors in making a moral difference.

Table 9 : Effect of bacterial and bocache fertilizer and their interactions on the content of the leaf of the vinca plants from Cathranthine (ppm)

Average *Azosper.*	*Bokashi*(%)					*Azosper.*
	40	**30**	**20**	**10**	**0.0**	
252.25	331.62	305.84	243.10	203.94	176.74	–
709.68	851.88	871.60	779.36	738.99	306.57	+
	591.75	588.72	511.23	471.47	241.66	Average *Bokashi*
	interferance	*Bokashi*		*Azosperillium* spp.		L.S.D.
	34.50	24.40		15.43		0.05

Discussion

It may explain the reason for the significant increase in the content of the leaves of the plant from the medically effective compounds to the role of the bacterial fertilizer *Azosperillium* in supplying the plant with the nutrients it needs to encourage growth, development and facilitate its absorption by the root system through its contribution in converting the non-ready images of absorption into accessible forms for absorption. In addition to its ability to provide the necessary growth regulators for growth, it also contributes to air nitrogen emissions through its symbiotic and non-symbiotic coexistence with the host plant roots (Chen, 2006). The metabolic and physiological activities of the plant are affected by the bacterial activity reflected in its growth through Providing the seedlings with sufficient quantities of nitrogen and increasing the readiness of phosphorus and potassium (Ali and Al-Jazuri, 2011), in addition to stimulating the photosynthesis process that increases the amount of carbohydrates and this is reflected directly in the increase in the active substance (Ali and Majeed, 2016), as mentioned by (Van Der Heijden *et al.*, 2002). The addition of bacterial fertilizer increases the alkaloids for its ability to increase the plant's nitrogen-stimulating content to increase the production of alkaloids. As for Chitosan (Ahmadi, 2015) mentioned that this compound has recently started to be widely used in increasing secondary metabolism compounds of the plant, including alkaloids, in addition to stimulating growth, in addition to its entry into the biological synthesis pathway of internal hormones (Auxin) as it enhances the production of the compound Tryptophan IAA (Youssef, 2016) is the initiator of biotin, and (Samarfard and Kadir, 2014) have confirmed that chitosan is an environmentally friendly carbohydrate that is often used to stimulate the growth of many plant species. (El-Hadrami, 2010) also indicated that chitosan works to early germination process as it improves the root system indicators in increasing the number and length of roots and their dry weights and thus improving the strength and growth of the seed and increasing its resistance against the various stresses it faces, as (Behboudi, 2018) confirmed that chitosan is used It is widely used in agricultural applications for its important role in protecting plants from different stresses, stimulating plant growth, antioxidant, speeding germination processes and improving economic output, as Abdel-Aziz *et al*. (2018) concluded that foliar spray of chitosan on the vegetative system of plants is easily absorbed from The way of external skin This leaves causes increased growth and production of many plants. As for the Bukashis, through the results shown in tables (5-9), the reason for the superiority of alkaloids may be attributed to its role in treating problems that may exist in the soil of the pot, including levels of organic matter, low fertility, limited humidity, high soil temperatures in summer, and increased

porosity with its ability to supply the plant with nutrients as a result Mineralizing its organic matter and granting the necessary energy needed by microorganisms (Fadillah *et al*., 2015), in addition to its role in providing many elements necessary for growth, including the major elements NPK with the production of a group of organic and amino acids, vitamins and antibiotics. He needs the plant during its life cycle (Charles, 2012). Lima *et al*. (2015) concluded in her study that adding the boccashi to the soil was a suitable alternative to increase its fertility as a source of organic matter and significantly improved its properties due to its chemical and biological properties that contribute to achieving positive effects on plant growth, as it considered it an effective tool for processing the necessary elements of the plant, as well Its high ability to provide and exchange important cations and increase the number of beneficial organisms in the soil while reducing the density of pathogenic organisms. As for the effect of salinity on the increase of Vib and Cath alkaloids (Table 3 and 5) to the role of salt NaCl in increasing the negativity of the osmotic potential of the plant cell, it plays an important role in increasing the rate of photosynthesis and thus is reflected directly in the increase of secondary metabolism compounds in reaction to the plant against the stress on it to protect itself Of the effects of salinity, including harmful free radicals of the plant (Zhou and Wu, 2006), and sodium ions activate the enzymes that stimulate the formation of alkaloids (Evans, 2002).

We conclude from the study that the addition of environmentally friendly factors used in the research experiment had a significant effect which was positively reflected in the increase in the concentration of medicinally active substances in the leaves of vinca plants and their reduction of the harmful effect of the risk of saline tension according to the study conditions.

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