

Effects of foliar spray of Boron Nanoparticles and Tillage system on production Medical active substances and chemical structure in Alfalfa (Medicago sativa L.)

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Abstract

Field experiment conducted at two summer season 2020-2021 and 2021-2022 on clay sandy soil in Al-Diwanyia. The experiment was design as Complete Randomized Block Design with three replications arranged for split-pilot design, and Least Significant differences $t_{0.05}$ the main treatment contend two level of Tillage system (10, 25)cm within their four levels of Boron Nanoparticles (BNPs)(0 , 10 , 20 , 30) mg.l⁻¹. and irrigated every 3 days I took sample of soil before planting to analysis it and to learn physical and chemical properties table 1. Alfalfa Seeds sowing at rate 20 kg.ha⁻¹(1 cm depth) at 1/4, after 7 months I took a samples to measure . The results showed all factors and interactions were significant effect and produce three medical active substances Oxalic acid , Methypyridazin, Benzaldehyde and protein , carbohydrates ,Fats , total chlorophyll , forage yield and their max values respectively(2.67 , 2.5 , 2.58 , (٢١.٤١) , 5.٥ , 1.02)% , 2.7 mg. 2g⁻¹ , 1.79 T,ha⁻¹) in interaction 25 cm and 30 mg.l⁻¹ while the min values respectively (0.02 , 0.02 , 0.02 , (18.8 , 4.83 , 0.78)% , 2.38 mg.2g⁻¹ and 1.33 T.ha⁻¹) in the interaction of 0 kg.ha⁻¹ and 0 mg.l⁻¹ BNPs .

Keywords : Tillage system , Foliar spray , Alfalfa , Nano B.

I. Introduction

Alfalfa one of main sources of Forage legume of animals diet in intercropping system[1] .also one of the medicinal plants [4] also Bio remediation because ability to remove heavy metals like Cadmium from soil. [9] also main sources of allelopathic which used in biological control[3]. Deep tillage system increased growth of alfalfa than the shallow tillage system (2 inches) . [10] . treatment 15 cm tillage system will raised soil temperature and water dynamic and enhance growth of crops[14] Foliar spray of 20PPM of BNPs and 200 PPM of Zn NPs increased fruits yield of Olive tree [6] This study aimed to learn Effects of foliar spray of BNPs and Tillage system on production Medical active substances in alfalfa.

II. Material and methods:

Seeds of Alfalfa inoculated with *Rhizobium meliloti* L.by imbibition seeds with the Rhizobium solution[8] *Rhizobium* culture's prepare from crushed sterile old root nodule with one drop of distal water then incubated at 30 °C for 3-7 days [13] So as to stimulate bio fertilizers also all treatments fertilized with 20 kg.ha⁻¹ Urea (47%N)to stimulate *nif* H gene it responsible on nitrogenase formation [16] Add 200mg seeds powder in glass test tube then closed by cork then injected absolute methanol by syringe and mixing at 10min.Then store at 6h in dark place then faltered 4.5μ and Iam add 1ml hexane,(100%) then analysis by GC-Mass.. Analysis of fats by dissolved 10 g of dry leaves powder with 10 ml Hexane 100%and inter to soxhlet .While analysis of carbohydrates depend on [7] other measures [2]

Tabl(1) showed analysis of soil before planting

Soil		
Value	Unite	Properties
7.42	-----	Soil PH
7.53		Water PH
14.5	(μS/cm)	Electrical conductivity
4.26	g.kg ⁻¹ of soil	Organic matter
73.21	mg.kg ⁻¹ of soil	A voluble nitrogen
41.75		A voluble phosphor
43		A voluble potassium



265	g.kg ⁻¹ of soil	Sand
175.4		Silt
559.6		Clay
Sandy – clay soil		Texture

III. Results and Discussion:

Leaves Protein percent %: P-LPL-[-; / - \)

Table(2) showed significant effect of 25 cm Tillage system on protein percent% max value(20.95%) of Alfalfa because increased soil aeration [12] lead to increased alkaline to form good environment to Rhizobium enhance root nodules which responsible on nitrogen fixation this results in line with [17] also showed significant effect of Nano BNPs fertilizers on protein percent of Alfalfa max value(20.74%) in treatment 30 mg.l⁻¹ because of increased Nano element passed through plasma membrane it is very small size and increased activity of nitrogenase in roots nodules [6]this is in line with[11 , 18] when BNPs as Nanozyme stimulate abiotic polypeptide synthesis. also showed significant effect of interaction of Nano BNPs and Tillage system max value(21.41%) in 25 cm Tillage system and 30 mg.l⁻¹ BNPs because of roles of BNPs as stimulator to protein synthesis enzymes and nitrogenase enzyme this accepted with[6 , 11] the differences between years depend on wind speed which increased soil plant atmospheric continuous and increased up take of elements and stem diameter. This accepted with[5]

Average Tillage system effect	Level of B NPs mg.l ⁻¹				Tillage system
	30	20	10	0	
20.61	21.08	20.7	20.27	20.39	Tillage system 25 cm
19.32	19.72	19.42	19.36	18.8	Tillage system 10 cm
LSD a = 0.06	20.4	20.06	19.82	19.6	Average of BNPs effect
	LSD a*b= 0.54				LSD b= 0.44

Average Tillage system effect	Level of B NPs mg.l ⁻¹				Tillage system
	30	20	10	0	
20.95	21.41	21.03	20.61	20.75	Tillage system 25 cm
19.74	20.06	19.77	19.83	19.31	Tillage system 10 cm
LSD a= 0.08	20.74	20.4	20.22	20.03	Average of BNPs effect
	LSD a*b= 0.53				LSD b= 0.43

Leaves Carbohydrates percent %: - \)

Table(3) showed significant effect of 25 cm Tillage system on Carbohydrates percent% max value(5.38%) of Alfalfa because increased soil aeration [12] lead to increased alkaline to form good environment to Rhizobium enhance root nodules which responsible on nitrogen fixation this results in line with [17] also showed significant effect of Nano BNPs fertilizers on Carbohydrates percent of Alfalfa max value(5.31%) in treatment 30 mg.l⁻¹ because of increased Nano element passed through plasma membrane it is very small size and increased activity of nitrogenase in roots nodules and photosynthesis and photorespiration and other essential processes [6]this is in line with[11 , 18] when BNPs stimulate abiotic polypeptide synthesis also showed significant effect of interaction of Nano BNPs and Tillage system max value(5.5%) in 25 cm Tillage system and 30 mg.l⁻¹ BNPs because of roles of BNPs as stimulator to Fats synthesis enzymes and nitrogenase enzyme this accepted with[6 , 11] the differences between years depend on wind speed which increased soil plant atmospheric continuous and increased up take of elements and stem diameter. This accepted with[5]

Average Tillage system effect	Level of B NPs mg.l ⁻¹				Tillage system
	30	20	10	0	
4.96	5.07	5.2	5.13	4.91	Tillage system 25 cm



4.66	4.76	4.98	4.88	4.63	Tillage system 10 cm
	4.91	4.68	4.59	4.77	Average of BNPs effect
LSD a = 0.15	LSD a*b= 0.15				LSD b= 0.1
effect Tillage system and BNPs on leaves Carbohydrates % in Alfalfa at 2021-2022					
Average Tillage system effect	Levels of BNPs				Tillage system
	30	20	10	0	
5.38	5.5	5.4	5.29	5.32	Tillage system 25 cm
5.01	5.13	5.06	5.04	4,83	Tillage system 10 cm
LSD a= 0.58	5.31	5.23	5.16	5.08	Average of BNPs effect
	LSD a*b= 0.46				LSD b= 0,26

Leaves Fats percent %: -۳

Table(4) showed significant effect of 25 cm Tillage system on Fats percent% max value(0,98%) of Alfalfa because increased soil aeration [12] lead to increased alkaline to form good environment to Rhizobium enhance root nodules which responsible on nitrogen fixation this results in line with [17] also showed significant effect of Nano BNPs fertilizers on Fats percent of Alfalfa max value(0,96%) in treatment 30 mg.l⁻¹ because of increased Nano element passed through plasma membrane it is very small size and increased activity of nitrogenase in roots nodules and photosynthesis and photorespiration and other essential processes [6] this is in line with [11 ,18] when BNPs stimulate as Nanozyme abiotic polypeptide synthesis also showed significant effect of interaction of Nano BNPs and Tillage system max value(1.2%) in 25 cm Tillage system and 30 mg.l⁻¹ BNPs because of roles of BNPs as stimulator to Fats synthesis enzymes and nitrogenase enzyme this accepted with [6 , 11] the differences between years depend on wind speed which increased soil plant atmospheric continuous and increased up take of elements and stem diameter. This accepted with [5]

Table (4) effect of Tillage system and BNPs on Fats % in Alfalfa at 2020-2021					
Average Tillage system effect	Levels of BNPs mg.l ⁻¹				Tillage system
	30	20	10	0	
0.94	0.98	0.95	0.92	0.92	Tillage system 25 cm
0.82	0.87	0.84	0.81	0.78	Tillage system 10 cm
	0.92	0.89	0.86	0.85	Average of BNPs effect
LSD a = 0.039	LSD a*b= 0.039				LSD b= 0.028
effect of Tillage system and BNPs on Fats % in Alfalfa at 2021-2022					
Average Tillage system effect	Level of B Nanoparticles mg.l ⁻¹				Tillage system
	30	20	10	0	
0.98	1.02	0.99	0.96	0.95	Tillage system 25 cm
0.87	0.91	0.88	0.87	0.82	Tillage system 10 cm
	0.96	0.93	0.91	0.88	Average of BNPs effect
LSD a= 0.04	LSD a*b= 0.035				LSD b= 0.023

Oxalic acid : -٤

Table(5) showed significant effect of 25 cm Tillage system on Oxalic acid max value(1.99) of Alfalfa because increased soil aeration [12] lead to increased alkaline to form good environment to Rhizobium enhance root nodules which responsible on nitrogen fixation this results in line with [17] also showed significant effect of Nano BNPs fertilizers on Oxalic acid of Alfalfa max value(2.65) in treatment 30 mg.l⁻¹ because of increased Nano element passed through plasma membrane it is very small size and increased activity of nitrogenase in roots nodules and photosynthesis and photorespiration and other essential processes [6] this is in line with [11 , 18] when BNPs as Nanozyme stimulate abiotic polypeptide synthesis also showed significant effect of interaction of Nano BNPs and Tillage system max value(2.67) in 25 cm Tillage system and 30 mg.l⁻¹ BNPs because of roles of BNPs as stimulator to Oxalic acid synthesis enzymes and nitrogenase enzyme this accepted with [6 , 11] the differences between years



depend on wind speed which increased soil plant atmospheric continuous and increased up take of elements and stem diameter. This accepted with[5]

Table (5) effect Tillage system and BNPs on Oxalic acid in Alfalfa at 2020-2021					
Average Tillage system effect	Levels of BNPs mg.l ⁻¹				Tillage system
	30	20	10	0	
1.64	2.21	2.18	2.16	0.02	Tillage system 25 cm
1.61	2.17	2.14	2.13	0.02	Tillage system 10 cm
	2.19	2.16	2.14	0.02	Average of BNPs
LSD a = 0.006	LSD a*b= 0.02				LSD b= 0.02
effect of Tillage system and BNPs on Oxalic acid in Alfalfa at 2021-2022					
Average Tillage system effect	Levels of BNPs mg.l ⁻¹				Tillage system
	30	20	10	0	
1.99	2.67	2.64	2.62	0.02	Tillage system 25 cm
1.96	2.63	2.6	2.59	0.02	Tillage system 10 cm
	2.65	2.62	2.6	0.02	Average of BNPs
LSD a= 0.006	LSD a*b= 0.02				LSD b= 0.02

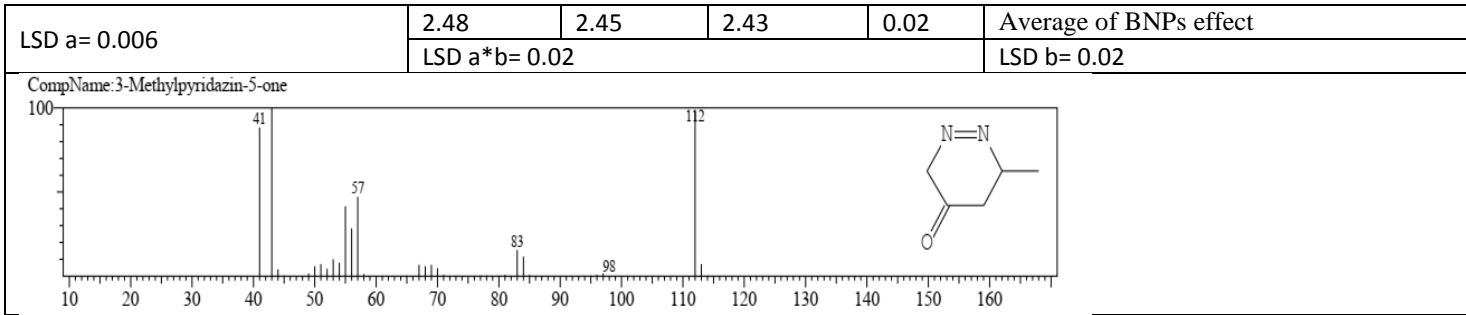
CompName: Oxalic acid, dicyclobutyl ester

Methypyridazin:

Table(6)showed significant effect of 25 cm Tillage system on Methypyridazin max value(1.86) of Alfalfa because increased soil aeration [12] lead to increased alkaline to form good environment to Rhizobium enhance root nodules which responsible on nitrogen fixation this results in line with [17] also showed significant effect of Nano BNPs fertilizers on Methypyridazin of Alfalfa max value(2.48) in treatment 30 mg.l⁻¹because of increased Nano element passed through plasma membrane it is very small size and increased activity of nitrogenase in roots nodules and photosynthesis and photorespiration and other essential processes [6]this is in line with[11 , 18] when BNPs as Nanozymes stimulate abiotic polypeptide synthesis also showed significant effect of interaction of Nano BNPs and Tillage system max value(2.5) in 25 cm Tillage system and 30 mg.l⁻¹ BNPs because of roles of BNPs as stimulator to Methypyridazin synthesis enzymes and nitrogenase enzyme this accepted with[5 , 11] the differences between years depend on wind speed which increased soil plant atmospheric continuous and increased up take of elements and stem diameter. This accepted with[5]

Table (6) effect Tillage system and BNPs on Methypyridazin at 2020-2021					
Average Tillage system effect	Levels of BNPs mg.l ⁻¹				Tillage system
	30	20	10	0	
1.4	2.14	1.72	1.71	0.02	Tillage system 25 cm
1.17	1.58	1.55	1.53	0.02	Tillage system 10 cm
	1.86	1.63	1.62	0.02	Average of BNPs effect
LSD a = 0.1	LSD a*b= 0.2				LSD b= 0.17
effect of Tillage system and BNPs on Methypyridazin in Alfalfa at 2021-2022					
Average Tillage system effect	Levels of BNPs mg.l ⁻¹				Tillage system
	30	20	10	0	
1.86	2.5	2.47	2.45	0.02	Tillage system 25 cm
1.83	2.46	2.43	2.42	0.02	Tillage system 10 cm

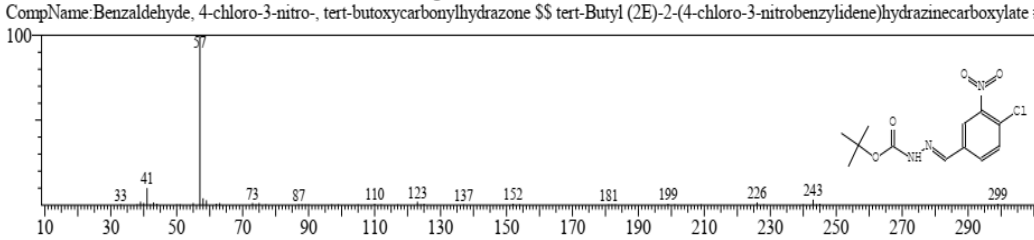




Benzaldehyde .I

Table(7) showed significant effect of 25 cm Tillage system on Benzaldehydemax value(1.94) of Alfalfa because increased soil aeration [12] lead to increased alkaline to form good environment to Rhizobium enhance root nodules which responsible on nitrogen fixation this results in line with [17] also showed significant effect of Nano BNPs fertilizers on Benzaldehyde of Alfalfa max value(2.59) in treatment 30 mg.l⁻¹ because of increased Nano element passed through plasma membrane it is very small size and increased activity of nitrogenase in roots nodules and photosynthesis and photorespiration and other essential processes [6] this is in line with [11, 18] when BNPs stimulate as Nanozyme able on abiotic polypeptide synthesis also showed significant effect of interaction of Nano BNPs and Tillage system max value(2.61) in 25 cm Tillage system and 30 mg.l⁻¹ BNPs because of roles of BNPs as stimulator to Benzaldehydesynthesis enzymes and nitrogenase enzyme this accepted with [6, 11] the differences between years depend on wind speed which increased soil plant atmospheric continuous and increased up take of elements and stem diameter. This accepted with [5]

Table (7) effect Tillage system and BNPs on Benzaldehyde in Alfalfa at 2020-2021					
Average Tillage system effect	Levels of BNPs mg.l ⁻¹				Tillage system
	30	20	10	0	
1.63	2.19	2.16	2.15	0.02	Tillage system 25 cm
1.61	2.16	2.13	2.12	0.02	Tillage system 10 cm
LSD a = 0.006	2.17	2.14	2.13	0.02	Average of BNPs
	LSD a*b= 0.028				LSD b= 0.023
effect of Tillage system and BNPs on Benzaldehyde in Alfalfa at 2021-2022					
Average Tillage system effect	Levels of BNPs mg.l ⁻¹				Tillage system
	30	20	10	0	
1.94	2.61	2.58	2.56	0.02	Tillage system 25 cm
1.91	2.57	2.54	2.52	0.02	Tillage system 10 cm
LSD a= 0.003	2.59	2.56	2.54	0.02	Average of BNPs
	LSD a*b= 0.02				LSD b= 0.018



7- Total Chlorophyll :

Table(8) showed significant effect of 25 cm Tillage system on Total Chlorophyll value(2.66) of Alfalfa because increased soil aeration [12] lead to increased alkaline to form good environment to Rhizobium enhance root nodules which responsible on nitrogen fixation this results in line with [17] also showed significant effect of Nano BNPs fertilizers on Total Chlorophyll Alfalfa



max value(2.64) in treatment 30 mg.l⁻¹ because of increased Nano element passed through plasma membrane it is very small size and increased activity of nitrogenase in roots nodules and photosynthesis and photorespiration and other essential processes [6]this is in line with[11 , 18] when BNPs stimulate as Nanozyme abiotic polypeptide synthesis also showed significant effect of interaction of Nano BNPs and Tillage system max value(2.7) in 25 cm Tillage system and 30 mg.l⁻¹ BNPs because of roles of BNPs as stimulator to Total Chlorophyll enzymes and nitrogenase enzyme this accepted with[6 , 11] the differences between years depend on wind speed which increased soil plant atmospheric continuous and increased up take of elements and stem diameter. This accepted with[5]

Table (8) effect of Tillage system and BNPs on total chlorophyll mg. 2g ⁻¹ leaves fresh weight in Alfalfa at 2020-2021					
Average Tillage system effect	Levels of BNPs mg.l ⁻¹				Tillage system
	30	20	10	0	
2.36	2.4	2.37	2.34	2.33	Tillage system 25 cm
2.24	2.28	2.25	2.24	2.2	Tillage system 10 cm
	2.34	2.31	2.29	2.26	Average of BNPs effect
LSD a = 0.04	LSD a*b= 0.03				LSD b= 0.02
effect of Tillage system and BNPs on total chlorophyll mg. 2g ⁻¹ leaves fresh weight in Alfalfa at 2021-2022					
Average Tillage system effect	Levels of BNPs mg.l ⁻¹				Tillage system
	30	20	10	0	
2.66	2.7	2.67	2.64	2.63	Tillage system 25 cm
2.55	2.59	2.56	2.55	2.51	Tillage system 10 cm
	2.64	2.61	2.59	2.57	Average of BNPs effect
LSD a= 0.04	LSD a*b= 0.03				LSD b= 0.02

Forage yield (Ton. ha⁻¹):

Table(9) showed significant effect of 25 cm Tillage system on Forage yield value(1.75) of Alfalfa because increased soil aeration [12] lead to increased alkaline to form good environment to Rhizobium enhance root nodules which responsible on nitrogen fixation this results in line with [17] also showed significant effect of Nano BNPs fertilizers on Forage yield Alfalfa max value(1.73) in treatment 30 mg.l⁻¹ because of increased Nano element passed through plasma membrane it is very small size and increased activity of nitrogenase in roots nodules and photosynthesis and photorespiration and other essential processes [6]this is in line with[11 , 18] when BNPs as Nanozyme stimulate abiotic polypeptide synthesis also showed significant effect of interaction of Nano BNPs and Tillage system max value(1.79) in 25 cm Tillage system and 30 mg.l⁻¹ BNPs because of roles of BNPs as stimulator to Forage yield enzymes and nitrogenase enzyme this accepted with[6 , 11] the differences between years depend on wind speed which increased soil plant atmospheric continuous and increased up take of elements and stem diameter. This accepted with[5]

Table (9) effect of Tillage system and BNPs on Forage yield(Ton. ha ⁻¹) in Alfalfa at 2020-2021					
Average Tillage system effect	Levels of BNPs mg.l ⁻¹				Tillage system
	30	20	10	0	
1.49	1.54	1.5	1.47	1.45	Tillage system 25 cm
1.37	1.233	1.38	1.37	1.33	Tillage system 10 cm
	1.41	1.44	1.42	1.39	Average of BNPs effect
LSD a = 0.04	LSD a*b= 0.03				LSD b= 0.02
effect of Tillage system and BNPs on Forage yield(Ton ha ⁻¹) in Alfalfa at 2021-2022					
Average Tillage system effect	Levels of BNPs mg.l ⁻¹				Tillage system
	30	20	10	0	
1.75	1.79	1.76	1.73	1.73	Tillage system 25 cm
1.64	1.68	1.65	1.63	1.6	Tillage system 10 cm
	1.73	1.7	1.68	1.66	Average of BNPs effect
LSD a= 0.037	LSD a*b= 0.03				LSD b= 0.019



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