Efficacy of Some Organic Amendments for the Control of Stem and Bulb Nematode, *Ditylenchus dipsaci* (Kühn) Filipjev on Garlic (*Allium sativum*)

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Abstract

A greenhouse experiment was conducted to evaluate effect of some soil amendments, i.e; fresh chopped leaves or dry leaves powder of *Datura stramonium*, *Peganum harmala* or *Tagetes minuta*, poultry and sheep manure in controlling the stem and bulb nematode, *Ditylenchus dipsaci* on garlic (*Allium sativum*) as compared to the nematicide, Vydate® (Oxamyl). All applied treatments significantly (P≤ 0.05) reduced final population of *D. dipsaci* except in treatment of sheep manure at lowest dosage rate, which significantly increased the population in comparison to that of the check. Efficacy of the treatments differed according to the type of organic soil amendment, dosage rate and type of application. Dry leaves powder of *Peganum harmala* was the most effective in reducing nematode counts in soil and garlic bulbs at the different rates, followed by fresh chopped leaves of *Datura stramonium* and *T. minuta* while, the lowest % reduction in such nematode parameters was recorded in case of soil amended with sheep manure followed by dry leaves powder of *Datura stramonium*. As for plant growth criteria the results reveal that most tested materials improved plant growth criteria of shoot, root and bulb compared to the untreated controls.

Keywords: *Ditylenchus dipsaci*, nematicides, botanical amendments, organic manures, garlic.

Introduction

The stem and bulb nematode, *Ditylenchus dipsaci* (Kühn) Filipjev is a serious pest of commercial garlic and onion in the colder regions. In temperate regions it is among the plant-parasitic nematodes of greatest economic impact. *D. dipsaci* is known to attack over 450 different plant species, including many weeds. However, it occurs in more than ten biological "races" some of which have a limited host range.

In Yemen, garlic and onion are the major hosts of *D. dipsaci*, causing complete failure of host crops when neglecting control procedures **(Awadh et al., 2008).** *D. dipsaci* infections can arise from planting in nematode-infested soil or more commonly from planting infected garlic seed cloves. Damages by *D. dipsaci*

are usually necrosis or rotting of bulbs, swellings and distortion of aerial plant parts, leaf yellowing and death of young plants. Chemical nematicides are effective in management of nematode problems, but their hazardous effect on human health, environment, ground water contamination and non-target organisms could limit its use **(Whitehead, 1998; Giannakou *et al*., 2004; Tsay*et al.*, 2004; Anastasiadis *et al*., 2008 and Washira *et al*., 2009).**

This has encouraged scientists to search for alternative sources of effective, cheap, eco-friendly and harmless methods for nematode control **(Noling and Becker, 1994 and Chitwood, 2003).** Plant extracts of some plants have been tested for nematicidal activity against *D. dipsaci in vitro* and in soil **(Insunza, 1988 and 1994; Insunza and Valenzuela, 1995; Awadh *et al*., 2008; Zouhar *et al.,* 2009 and Hassan, *et al.*, 2015).** Soil amendments with fresh chopped or dry ground leaves of plants, poultry manure and sheep manure have been successfully used for the control of plant-parasitic nematodes and improve plant growth. However, the most researches were focused on the root-knot nematodes, *Meloidogyne spp.* **(Muller and Gooch, 1982; Alam, 1990; Akhtar and Mahmood, 1996; Nahar *et al*., 1996; Akhtar and Malik, 2000; Kheir, *et al.*, 2000; Sundararaju *et al*., 2003; Verma, andKhan, 2004; Abdul Latif, *et al.*, 2006; Wani, 2006; Ahmad, *et al*., 2007; Ibrahim, *et al.*, 2007; Radwan, et *al*., 2007; Rather, et al., 2008; Ibrahim and Traboulsi, 2009; Kagai, 2009; Hussain, *et al*., 2011; Karmani *et al*., 2011; Moosavi, 2012; Parihar *et al*., 2012; Ishaku and Ahaji, 2013; Ojo and Umar, 2013; Youssef and Lashein, 2013 and Saeed and Shawkat, 2014).**

The objective of this research was to evaluate the efficacy of soil organic amendments with fresh chopped leaves and dry leaves powder of three plant species (*Datura stramonium, Peganum harmala* and *Tagetes minuta*), poultry and sheep manures on the control of stem and bulb nematode, *D. dipsaci* infecting garlic under greenhouse conditions.

Materials and Methods

Collection and Preparation of Leaves and Manures:

Leaves of *Datura stramonium, Peganum harmala* and *Tagetes minuta* were separately collected during summer of 2011 from different plants of different locations at Sana'a University, Yemen. The leaves were air-dried on laboratory benches in the Department of Plant Protection for 2 weeks at 20-30°C. Then they were ground separately into fine powders with Thomas Wiley laboratory mill and electric Warring blinder and stored in a sealed container until using. The fresh leaves were washed in tap water and then finely chopped into very fine pieces. Dried chicken and sheep manures were obtained from research farm of the Faculty of Agriculture, ground and sieved over a 0.5-cm sieve.

Preparation of Inoculum:

The stem and bulb nematode inocula were obtained from bulbs of a highly infested commercial garlic field, which were extracted as previously described by **Southey (1986**) and OEPP/EPPO **Bulletin (2008).** The extracted nematodes were identified and then counted using a stereoscopic microscope.

Greenhouse Experiment:

Balady garlic cloves with uniform size were individually planted in 15-cm. diameter plastic pots filled with steam sterilized loamy sand soil (3:1, w:w). Plants were grown at natural photoperiod in a greenhouse for 2 weeks at a constant temperature of 20°C ± 5 and the uniform plants were then selected for treatment application later. Two weeks old garlic plants were inoculated with 500 nematodes of mixed life stages by pipetting 5 ml aqueous suspensions of the nematodes into 5 holes in the soil around the roots; and the holes were immediately filled with moist soil. Five plants were kept without inoculation to serve as a healthy check treatment. Ten days after inoculation 5, 10 and 15g (equilvalent to 5.7, 11.4 and 17.1 t/ha, respectively) of fresh chopped leaves or dry leaves powder were incorporated on top of soil around the base of each plant. Poultry and sheep manures were applied at the rate of 5, 10 and 15 g/pot; while Vydate® (oxamyl) was used at the rate of 1gm/ pot as a comparable treatment. Some pots of nematode-inoculated plants were kept without materials application to serve as a check. Each treatment was replicated 5 times and all pots were arranged in a randomized block design on a greenhouse bench at temperature degrees of $20^{o}C \pm 5$. All plants received similar treatments of fertilizing and irrigation. Sixty days after inoculation, the experiment was ended and all plants were rooted-off and nematode counts in both soil and plants were estimated. Plant growth criteria on the basis of shoot and root lengths; and shoot, root and bulb fresh weights were measured. Data were subjected to analysis of variance and means were compared at $P \leq 0.05$ using SPSS software version 21.

Results

Data in Table (1) indicate that all applied treatments significantly (P≤0. 05) reduced final population of *D. dipsaci* except that of sheep manure at the lowest dosage rate, which significantly increased the population in comparison to that of the check. Efficacy of the treatments differed according to the type of organic soil amendment, dosage rate and method of application. Generally, by increasing the amendment dosage rate, the efficacy was also increased. Dry leaves powder of *P. harmala* was more effective in reducing the nematode population than fresh chopped leaves, but the reverse was true for *D. stramonium* and *T. minuta.* Dry leaves powder of *P. harmala* was the most effective in reducing nematode counts in soil and garlic bulbs with all rates, followed by fresh chopped leaves of *D. stramonium* and *T. minuta.*

Table (1): Effect of organic soil amendments on population of *Ditylenchus dipsaci* infecting garlic plants under greenhouse conditions.

Treatments	Dose	Nematode counts					
	gm.	In Soil	%Reduction	In Plant	%Reduction	Final population	Build up
Datura stramonium (F)	5	274.0 efg	44.0	179.6 abcd	89.7	453.6 bcde	0.9
	10	206.2 cdef	57.8	228.1 abcd	86.9	434.3 bcd	0.9
	15	104.8 abcd	78.6	135.0 abc	92.2	239.8 abc	0.5
***Datura stramonium* (D)**	5	559.3 jk	-14.4	680.2 efgh	60.9	1239.5 h	2.5
	10	421.5 hi	13.8	528.5 defg	69.6	950.0 gh	1.9
	15	323.7 fgh	33.8	233.4 abcd	86.6	557.1 cdef	1.1
Peganum harmala (F)	5	237.6 def	51.4	945.2 hi	45.7	1182.8 h	2.4
	10	398.0 ghi	18.6	440.0 bcde	74.7	838.0 efgh	1.7
	15	196.0 cdef	59.9	300.8 abcd	82.7	496.8 bcde	1.0
***Peganum harmala* (D)**	5	118.0 abcd	75.9	295.0 abcd	83.0	413.0 bcd	0.8
	10	0.0 a	100.0	208.1 abcd	88.0	208.1 abc	0.4
	15	0.0 a	100.0	0.0 a	100.0	0.0 a	0.0
***Tagetes minuta* (F)**	5	303.2 fgh	38.0	835.1 fghi	52.0	1138.3 h	2.3
	10	106.0 abcd	78.3	229.0 abcd	86.8	335.0 abcd	0.7
	15	52.2 ab	89.3	150.4 abcd	91.4	202.6 abc	0.4
Tagetes minuta (D)	5	244.0 def	50.1	877.2 ghi	49.6	1121.2 h	2.2
	10	196.4 cdef	59.8	718.7 efghi	58.7	915.1 fgh	1.8
	15	150.2 bcde	69.3	499.9 cdef	71.3	650.1 defg	1.3
Poultry manure	2	160.0 bcde	67.3	922.3 hi	47.0	1082.3 h	2.2
	4	78.2 abc	84.0	841.6 fghi	51.6	919.8 fgh	1.8
	6	89.0 abc	81.8	892.5 ghi	48.7	981.5 gh	2.0
Sheep manure	5	1114.0 l	-127.8	1931.6 j	-11.0	3045.6 k	6.1
	10	663.2 k	-35.6	1078.4 i	38.0	1741.6 i	3.5
	15	233.0 def	52.4	968.6 hi	44.3	1201.6 h	2.4
Oxamyl	1	0.0 a	100.0	97.4 ab	94.4	97.4 ab	0.2
Check		489.0 ij	0.0	1739.6 j	0.0	2228.6 j	4.5

Means followed by the same letter(s) within a column are not significantly different ($p \leq 0.05$) according to Duncan's multiple range test.

F= Fresh chopped leaves. D= Dry leaves powder.

While, the lowest % reduction in nematode counts was recorded in treatment of soil amended with sheep manure followed by those of dry leaves powder of *D. stramonium* and poultry manure. Insignificant differences were observed in nematode counts in soil between the check and those treated with fresh chopped leaves of *P. harmala* at modest dosage rate, and dry leaves powder of *D.*

stramonium at lowest and modest dosage rates. In contrast, sheep manure at lowest and modest dosage rates caused significant increase in nematode counts in soil.

Highest % reduction (100%) in nematode counts in soil was achieved by dry leaves powder of *P. harmala* at modest and highest dosage rates as well as Vydate® (Oxamyl). In general, all treatments significantly succeeded in reducing counts of nematode in garlic bulbs, except in treatment of sheep manure at lowest dosage rate. Likely, dry leaves powder of *P. harmala* at the highest dosage rate achieved 100% nematode reduction in garlic bulbs, followed by "Vydate®" (94.4%) and highest dosage rate of fresh chopped leaves of *D. stramonium* (92.2%) and then *T. minuta* (91.4%). Accordingly, the nematode final population and its rate of build-up of all treatments - except those of sheep manure at lowest dosage rate - were significantly differed from those of the check. Comparatively, all dosage rates of *P. harmala* dry leaves powder achieved the lowest rates of build-up (0.0, 0.4 and 0.8), followed by that of "Vydate®"(0.2). Almost, similar results were obtained by fresh chopped leaves of *T. minuta* (0.4 and 0.7) and *D. stramonium* (0.5 and 0.9) at highest and modest dosage rates. In contrast, sheep manure at different dosage rates caused the highest values of build up, (6.1, 3.5 and 2.4).

As for plant growth criteria, data presented in Table (2), reveal that most applied amendments improved growth of shoots, roots and bulbs of garlic. Relatively, remarkable increases were noticed in weights than in lengths of shoots than in roots. Significant increase in shoot fresh weight was obtained by most treatments except in case of fresh chopped leaves of *P. harmala* and sheep manure at different dosage rates, lowest dosage rate of dry leaves powder of *P. harmala* and *D. stramonium,* lowest dosage rate of fresh chopped leaves of *D. stramonium* and lowest and highest dosage rate of fresh chopped leaves of *T. minuta*. The highest % increase in shoot fresh weight was recorded in plants treated with fresh chopped leaves of *D. stramonium* at highest dosage rate (282.3%), followed by those of Vydate® (254.5%), dry leaves powder of *T. minuta* at modest dosage rate (248.4%) and poultry manure at modest dosage rate (209.7%).Shoot length was significantly increased in all treatments except in treatments of fresh chopped leaves of *P. harmala* at different dosage rates, dry leaves powder for *P. harmala* at highest dosage rate and sheep manure at modest dosage rate. The highest shoot length was achieved in plants treated with dry leaves powder of *D. stramonium* at highest dosage rate with 80 % increase, followed by Vydate® achieving 76.9% increase. Statistically, insignificant differences were noticed between such values and those of healthy control. With regard to garlic root parameters, root weight was more affected with the tested soil amendments than root length. All tested treatments significantly increased root weight except in case of fresh chopped leaves of *P. harmala* at lowest dosage rate.

Table (2): Effect of organic soil amendments on growth parameters of garlic plants infected with *Ditylenchus dipsaci* under greenhouse conditions.

Treatments	Dose	Shoot			Root				Bulb		
	gm.	Weight (gm)	Increase%	Length (cm)	Increase%	Weight (gm)	Increase%	Length (cm)	Increase%	Weight (gm)	Increase %
Datura stramonium (F)	5	2.5 bcdefg	108.2	33.6 bcdef	46.7	7.7 bcdefg	126.5	30.1 abc	7.9	1.2 bcde	500.0
	10	3.7 fghijk	196.8	35.2 bcdefg	53.7	8.3 cdefg	144.1	35.9 cdef	28.7	1.3 bcdef	550.0
	15	4.7 jk	282.3	38.8 defg	69.4	11.8 h	246.5	33.4 bcde	19.7	2.5 l	1150.0
Datura stramonium (D)	5	2.1 abcde	67.7	32.2 bcdef	40.6	6.1 bc	78.8	33.3 bcde	19.4	1.3 bcdefg	550.0
	10	2.6 cdefgh	111.3	37.4 cdefg	63.3	5.7 b	67.1	30.1 abc	7.9	1.0 bcd	400.0
	15	3.7 fghijk	195.2	41.1 fg	79.5	7.5 bcdefg	121.2	34.3 bcde	22.9	1.5 cdefgh	650.0
Peganum harmala (F)	5	1.0 a	-16.7	30.0 abcd	31.0	3.2 a	-7.1	26.0 a	-6.8	0.4 a	100.0
	10	1.1 a	-11.3	26.6 ab	16.2	5.6 b	64.1	33.3 bcde	19.4	0.7 ab	250.0
	15	2.5 bcdefg	98.4	30.5 abcd	33.2	7.1 bcde	107.6	30.7 abcd	10.0	1.0 bcd	400.0
Peganum harmala (D)	5	2.5 bcdefg	100.0	34.2 bcdef	49.3	9.7 fgh	185.9	36.8 def	31.9	1.8 fgh	800.0
	10	3.1 cdefgi	150.0	32.2 bcdef	40.6	9.8 gh	187.6	31.3 abcd	12.2	2.4 l	1100.0
	15	3.2 cdefgi	154.8	29.9 abcd	30.6	9.0 efg	165.3	33.4 bcde	19.7	1.9 ghi	850.0
Tagetes minuta (F)	5	2.0 abcde	58.1	34.9 bcdefg	52.4	9.0 efg	164.1	31.1 abcd	11.5	2.0 hi	900.0
	10	3.7 ghijk	201.6	37.2 cdefg	62.4	9.1 efg	167.1	38.5 ef	38.0	1.9 ghi	850.0
	15	2.5 bcdefg	103.2	35.6 cdefg	55.5	6.6 bcd	92.9	28.2 ab	1.1	2.1 hi	950.0
Tagetes minuta (D)	5	3.3 efghi	164.5	38.1 cdefg	66.4	7.4 bcdef	117.1	31.2 abcd	11.8	1.2 bcde	500.0
	10	4.3 ijk	248.4	38.4 cdefg	67.7	7.1 bcde	108.8	41.5 f	48.7	1.9 ghi	850.0
	15	3.5 fghij	182.3	38.7 defg	69.0	8.5 defg	150.0	30.0 abc	7.5	1.6 defgh	700.0
Poultry manure	2	3.2 defghi	161.3	35.6 cdefg	55.5	7.5 bcdefg	121.8	30.5 abcd	9.3	1.7 efgh	750.0
	4	3.8 hijk	209.7	35.4 bcdefg	54.6	7.6 bcdefg	124.7	30.8 abcd	10.3	1.8 fgh	800.0
	6	3.2 cdefghi	154.8	32.6 bcdef	42.4	7.3 bcde	115.3	31.5 abcd	12.9	1.6 defgh	700.0
Sheep manure	5	2.0 abcd	59.7	31.7 bcde	38.4	5.6 b	63.5	28.3 ab	1.4	1.0 bcd	400.0
	10	1.9 abc	53.2	29.4 abc	28.4	5.8 b	71.8	30.5 abcd	9.3	0.9 bc	350.0
	15	2.4 bcdef	93.5	33.4 bcdef	45.9	6.7 bcde	98.2	26.9 a	-3.6	1.0 bcd	400.0
Oxamyl	1	4.4 ijk	254.8	40.5 efg	76.9	7.1 bcde	108.8	31.4abcd	12.5	1.6 efgh	700.0
Check		1.2 ab	0.0	22.9 a	0.0	3.4 a	0.0	27.9 ab	0.0	0.2 a	0.0
Healthy		4.8 k	287.1	43.6 g	90.4	8.8 defg	160.0	35.1 cde	25.8	1.7 efgh	750.0

Means followed by the same letter(s) within a column are not significantly different (p=0.05) according to Duncan's multiple range test.

F= Fresh chopped leaves. D= Dry leaves powder.

The highest root fresh weight was recorded in the plants treated with fresh chopped leaves of *D. stramonium* at highest dosage rate by 246.5% increase followed by dry leaves powder of *P. harmala* at modest and lowest dosage rates with 187.6% and 185.9% increase, respectively; however, a such increase was 160% in case of healthy control. On the other hand, most tested soil amendments at different dosage rates caused insignificant % increase in root lengths except in case of modest dosage rate of fresh chopped leaves of *D. stramonium* and *T. minuta* and dry leaves powder of *T. minuta* and lowest dosage rate of dry leaves powder of *P. harmala.* All soil amendment treatments remarkably achieved the highest significant increase in bulb weights over the check in comparison to those of the other plant growth criteria with increase ranged between 100-1150%.

Discussion

Amending soil with fresh chopped leaves or dry leaves powder of the tested plants and poultry manure were very effective in suppressing stem nematodes in varying degree and enhanced growth and yield of garlic compared to the untreated control.

The nematode reproduction factor was decreased as the soil amendment dosage rate was increased. These results are in agree with those obtained by **Kheir *et al.*, (2000), Hosseininejad, (2004); Radwan, *et al.* (2007); Ntalli *et al.* (2010); Karmani *et al.* (2011); Cavoski *et al.* (2012); Onyeke and Akueshi (2012) and Saeed and Shawkat (2014).**

Fresh chopped leaves of *D. stramonium* and *T. minuta* were more effective than dry leaves powder in reducing nematode population. It is possible that the main plant compound responsible for the nematicidal effect as α-therthienyl in *Tagetes* species was destroyed during the preparing process of powder **(Wang *et al.*, 2007).** But the reverse was true for *P. harmala* and that may be attributed to amount increase of nematicidal compounds in case of dry leaves powder or to the slow and continuous release of the nematicidal compounds in soil. *P. harmala* achieved the strongest nematicidal activity against *D. dipsaci* on garlic under greenhouse conditions, followed by *D. stramonium* at different dosage rates in comparison to that of the check. This could be attributed to production of strong nematicidal substances like harmal's alkaloids include β-carboline as harmine, harmaline, harmalol, harmol and harman and quinazolines as vaccine and vasicinone **(El-Hassan *et al.*, 2013).** Active ingredients of *D. stramonium* are atropine, nicotine, hyoscyamine and scopolamine which are classified as deliriants, or anticholinergics **(Oduor- Owino, 1993 and Archana and Prasad, 2014)** and amending soil with leaves of such plant was effective against *M. incognita* **(Chattopadhyay, 1991; Radwan, *et al.*, 2007; Hussain, *et al.*, 2011 and Saeed and Shawkat, 2014).** The nematicidal activity of *T. minuta* can be attributed to its contents of E-tagetenone, jasmolins I and II, limonene, myrcene, beta-

caryophyuenc, cinerins I and II, α -terthienyl, bithienyl, 5(-ent-1-ol)-2,2-bithienyl, sigma-4, 22-dien-3-beta-ol, 5-(4-acetoxy-1-butenyl) -2,2-bithienyl, -butyl-2,20-bithienyl and 5-(3-buten-1-eynyl)-2,20-bithienyl **(Uhlenbroek and Bijloo, 1958 & 1959; Gommers and Bakker, 1988; El-Gengaihi *et al.*, 2001 and Archana and Prasad, 2014).** Amending soil with poultry manure significantly reduced nematode population and improved plant growth. This result is in agreement with the findings of **Kablanand Noe (1993), El-Zawahry (2000), Ibrahim and Ibrahim (2000), Maareg *et al.* (2000), Devi and Hassan (2002), Farahat *et al.* (2008), Karmani *et al.* (2011), Farahat *et al.* (2012) and Saeed and Shawkat (2014).**

Poultry manure at all dosage rates was better than sheep manure in controlling stem nematode and improving plant growth. These findings are in agreement with the work of **Miano (1999)** and **Karmani *et al.* (2011a&b).** The action of organic manures against soil nematodes is mainly attributed to its contents of volatile fatty acids, phenols, amino acids, ammonia, nitrites and gasses which released during decomposition enhancing soil populations of micro-organisms antagonistic to nematodes **(Badra, *et al.*, 1979; Mian and Rodrequez-Kabana, 1982; Lazarovits *et al.*, 2001; Oka and Pivonia, 2002 and Farahat *et al.*, 2008).**

The increase in growth of *D. dipsaci* infected garlic plants grown in treated soils compared to the untreated soils may be attributed partly to nematode control and to beneficial effects on soil nutrients, soil physical conditions and soil biological activity **(Akhtar and Malik, 2000; Ramesh *et al.*, 2009; Oka, 2010 and Moosavi, 2012).** Therefore, the use of fresh chopped leaves or dried leaves powder of the tested plants and poultry manure may gave promise tools to suppress nematode populations and may provide environmentally safe and economically cheap alternatives to chemicals. Further investigations are necessary to confirm their effectiveness under field conditions before final decision.

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الملخص العربي

تقييم تأثير إضافة بعض الأسمدة العضوية النباتية والحيوانية للتربة في مكافحة نيماتودا السوق والأبصال (دايتيلنكس ديبساسي) على نبات الثوم تحت ظروف الصوبة

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تم تقيم إضافة بعض الأسمدة العضوية كمفروم أوراق طازجة أو مطحون أوراق جافة لنباتات الداتورا *Datura stramonium*، الحرمل *Peganum harmala* والقطيفة *Tagetes minuta* وسبلة الدواجن والأغنام مقارنة بالمبيد النيماتودي الفايديت في مكافحة نيماتودا السوق والأبصال *Ditylenchus dipsaci* على الثوم. ولقد أظهرت النتائج أن كل المعاملات خفضت أعداد النيماتودا عدا سبلة الغنم عند الجرعة المنخفضة والتي زاد فيها أعداد النيماتودا معنويًا مقارنة بالشاهد، كما اختلفت كفاءة المعاملات طبقًا لنوع المواد العضوية وطريقة استخدامها وكذا الجرعة المضافة. سجل مطحون الأوراق الجافة للحرمل أعلى كفاءة خفض لأعداد النيماتودا في التربة ورؤوس الثوم عند كل الجرعات يليها مفروم الأوراق الطازجة لنباتي الداتورا والقطيفة بينما أعطى متخلف الغنم أقل نسبة خفض للنيماتودا يليه مطحون الأوراق الجافة لنبات الداتورا، ولقد حسنت معظم المعاملات النمو النباتي للأوراق، والجذور والرؤوس للثوم مقارنة بالشاهد الغير معامل.