EFFECT OF NITROGEN AND MANGANESE FOLIAR SPRAY ON GROWTH, YIELD, ACTIVE CONSTITUENTS IN Ambrosia maritima L. AND ITS HERB EXTRACT ON HYPERGLYCEMIA IN STREPTOZOTOCIN INDUCED DIABETIC RATS

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ABSTRACT

This study was conducted in two seasons 2002 - 2003 at Maryout Research Station, Alexandria Governorate, to study the effect of ammonium sulfate at 1000 ppm and 2000 ppm foliar spray plus three levels of manganese sulfate (0, 50, 100 ppm) on Ambrosia maritima L. plants. The results showed a significant increase in, plant height, fresh and dry weights per plant and herbage yield per feddan, as well as the active ingredients (ambrosin and damsin) as a result of using 2000 ppm ammonium sulfate plus 100 ppm manganese sulfate.

An aqueous and ethanolic extracts of Ambrosia maritima L. herb were tested for its effect on the glucose level in streptozotocin induced diabetic rats. The extracts showed a highly significant hypoglycemic effect on the experimental animals, after ten days. All the diabetic rats exhibited normal glucose level and ALT, AST and alkaline phosphatase enzymes returned to normal values after the treatments. The observed changes in glucose and enzyme levels in serum, suggest that the Ambrosia maritima L. herb extracts used in this study may represent an effective treatment for streptozotochin induced diabetes mellitus in rats.

Key words: Ambrosia maritima L., ammonium sulfate, diabetic hypoglycemia, medicinal plants, streptozotocin.

1.INTRODUCTION

Ambrosia maritima L. (Damsissa) belongs to the family Asteraceae (compositae) which is one of the largest families of the plant kingdom. It includes about 25000 species in over 15000 genra. Ambrosia maritima L. plant is a perennial dark green hairy herb, up to one meter height, with simple necked alternated lobed leaves (Tuckholm, 1974).

Sidky and El-Margawy (1997), found that the major constituents of Ambrosia maritima L. were the sesquiterpene lactones (ambrosin and damsin) which were found mainly in the leaves. The aqueous leaves extract is used in traditional medicine to treat gastrointestinal disturbances, (Bakhiet and Adam, 1996). Decoction of the plant is used for rheumatic pains, asthma, bilharziasis, diabetes and expelling kidney stones. Flowering branches are stimulant, stomachic, slightly astringent, emollient, vulnerary, diuretic and renal trouble (Loutfy 1983 and El-Shabrawy and Nada 1996). Also, Abou Basha et al., (1994), El-Ansary et al., (2000) and Allam (2000) found that ambrosin and damsin had a significant effect on controlling many kinds of snails in water canals.

El-Rab (2000), mentioned that the dry matter of *Ambrosia maritima* L. is used as a natural material to control nematodes appeared in plastic houses. Mohamed and Mortada (2001), indicated that, the aqueous - methanolic extract of *Ambrosia maritima* L. leaves may act as a hepato - protective and an anti - oxidant agent.

Many investigations were carried out dealing with nitrogen fertilization and its effect on growth and yield of medicinal and aromatic plants. [Tesi et al., (1995) and Cabrera and Devereaux, (1998)].

In addition, manganese plays an important role in plant life. It is considered as an essential nutrient. Hewitt and Smith (1975), found a clear indirect relationship between the influence of Mn²⁺ on photosynthesis and NO₂ reduction. Homann (1967), reported that chloroplasts are the most sensitive of all cell organelles to Mn²⁺ deficiency. Busslar (1958), obvoised that tissues suffering from Mn²⁺ deficiency have a small cell volume. Lindsay (1972), mentioned that, Mn nutrient application is very important to recover the deficiency of Mn²⁺ on plants especially grown under high soil pH conditions. Moreover, Lindsay (1972) stressed that the soluble Mn²⁺ decreases 100

fold for each unit increase in pH. So, the application of physiologically acid fertilizers, as (NH₄)₂SO₄ ammonium sulfate has a beneficial effect on Mn²⁺ uptake by plants. In biochemical functions Mn²⁺ resembles Mg²⁺, both ion species bridge ATP with the enzyme complex phosphokinases and phosphotransferase; Lehninger (1975). Manganese also in some way is involved in the oxidation - reduction processes in photosynthetic electron transport system. (Konrad and Kirkby 1979).

The aim of this investigation was to study the effect of nitrogen as fertilizer ammonium sulfate and Mn²⁺as manganese sulfate on growth, yield and active constituents of damsisa plants grown under calcarious soil at Maryout location, Alexandria Egypt. In addition hypoglycemia effect of *Ambrosia maritima* L. extract in streptozotocin induced diabetic rats was reported.

2. MATERIALS AND METHODS

The present investigation was carried out during two successive seasons (2002 and 2003) at Maryout Experimental Station, Alexandria, Governorate, Egypt.

Ambrosia maritima L. (Damsisa) seeds were obtained from the Medicinal and Aromatic Plants Department, Agricultural Research Center.

The seeds were sown in seedbeds on 1st of Nov. for both seasons. Uniform seedlings 45 day old and about 20 cm height were individually transplanted on rows 60 cm apart and the distance between hills was 50 cm (13333 plant / fed.),

The experiment was designed as a randomized complete block in three replicates. The experimental plot was (4 m x 2.70 m), had 36 plants and used as a replicate. The foliar spraying treatments were as follow:

- 1- Control (without fertilization).
- 2- 1000 ppm ammonium sulfate + 0 manganese sulfate.
- 3-1000 ppm ammonium sulfate plus 50 ppm manganese sulfate.
- 4- 1000 ppm ammonium sulfate plus 100 ppm manganese sulfate.
- 5- 2000 ppm ammonium sulfate + 0 manganese sulfate.
- 6- 2000 ppm ammonium sulfate plus 50 ppm manganese sulfate.

7-2000 ppm ammonium sulfate plus 100 ppm manganese sulfate.

All treatments were fertilized with phosphate and potassium dressing as the recommended rates. The plants were foliar sprayed with different concentrations (treatments) twice every season. The first spray was 15 days after transplanting and the second one was after 15 days from the first cut. Two cuts of *Ambrosia maritima* L.herb were taken every season. The first one on the 1st of June and the second on the 1st of Oct. in the two seasons.

The following data were recorded:

A- Vegetative growth

- 1- Plant height in (cm).
- 2- Total fresh and dry weights per damsisa plant (g) every cut.
- 3- Estimated fresh and dry herb yield of damsisa per feddan (Kg).

B- Active constituent contents:

Damsin and ambrosin percentages in dried powdered leaves were determined according to the methods described by Amin (1990).

The statistical analysis was carried out according to Snedecor and Cochran (1982), by computer SAS program.

2.1 Biological experiment

2.1.1 Animals

Male albino rats, Sprague – Dawley strain, weighing between $200-215\,$ g were used. They were obtained from Helwan breeding farm, Cairo Egypt. The animals were divided into four groups and housed individually in stainless steel cages with wire mesh bottoms in a room maintained at $25-30\,^{\circ}\text{C}$.

2.1.2 Preparation of ethanolic extract of damsisa leaves for the bioassay.

The biologically active substance was extracted with ethyl alcohol 70% (v/v).

Dried ground leaves (100 g) were covered with 500 ml of cold ethyl alcohol 70% (v/v) at room temperature for 2 days. The extract was filtered through whatman No. 1. The obtained clear extract was then evaporated (under vacuum) in order to remove the ethanol. The residue was dissolved in 500 ml of deionized water.

2.1.3 Experimental diets

Adult male albino rats were divided into four groups (eight animals each) and fed on a basal diet for eight days. This diet consisted of corn starch 70%, casein 10%, corn seed oil 10%, salt mixture 4%, vitamin mixture 1% and cellulose 5% (Compbell, 1961). The salt mixture used was that proposed by Higsted, et al., 1941 and the vitamin mixture described by Compbell, 1961.

The first group (8 rats) served as the control receiving neither streptozotocin (STZ) nor damsisa treatment. The second group (8 rats) was given damsisa extract without prior injection of streptozotocin to see the influence of damsisa extract on serum glucose level in normal rats. The third group (8 diabetic rats by intraperitoneal injection of a streptozotocin solution in a single dose of 50 g/Kg. b.w.) according to Szkudelski, (2001) was fed on basic diet, not receiving any treatment, but evaluated for serum glucose, and other blood analyses in order to compare with those of the herb (damsisa) treated rats.

The fourth group, (16 rats) was rendered diabetic by intraperitoneal injection of a streptozotocin solution; it was then divided into 2 subgroup. The first subgroup (8 streptozotocin induced diabetic rats after overnight fast) received damsisa extract by oral intubation in a dose (one ml / 100g.b.w.) daily for 10 days. At 2, 4, 6, 8 and 10 days of the treatments, serum glucose level was measured in each rat using the method of Trinder (1969). The second subgroup (8 diabetic rats) was treated the same as the first subgroup except that the dose of 2 ml / 100 g.b.w. was administered.

2.1.4 Analytical procedures

Blood samples were obtained from each rat from the orbital venous plexuses by capillary tube every 48 hour intervals for 10 days to determine blood glucose level. Blood samples were obtained from the portal vein and left to clot and centrifuged at 3000 rpm for 15 min to obtain serum.

Serum levels of transaminases (AST and ALT) were analyzed by enzymatic colorimetric procedure kits supplied by Biocon Diagnostik Gmb H, Burbach, Germany, according to Retiman and Frankel, (1957). Serum level of alkaline phosphatase was analyzed according to Kind and king (1954).

Uric acid and urea were determinated according to Caraway,

(1975) and creatinine according to Scirmeister, (1964).

Results are expressed as means SEM, and evaluated for significance by Student's (t) test *(Gomez and Gomez, 1984).

3.RESULTS AND DISCUSSION

3.1 Effect of nitrogen and manganese foliar spray on vegetative growth and yield of Ambroisa maritima L. plants.

3.1.1 Plant height

Data presented in Table (1) show that all foliar spray with N and Mn treatments caused a highly significant increase in plant height when compared with the control in both seasons. It is clear that plant height increased progressively as nitrogen and Mn²⁺ concentrations were increased in the two seasons.

The tallest damsisa plants were observed when plants were foliar sprayed with 2000 ppm ammonium sulfate plus 100 ppm manganese sulfate which reached 104.50 cm and 103.3 cm in the first and second seasons, compared with 64.90 cm and 68.90 cm obtained from control in the two seasons, respectively.

These results are in agreement with those obtained by Cabrera and Devereaux, (1998), who found that, application of Mn²⁺ on *Matricaria chamomilla* plants gave the tallest plants compared with untreated plants.

3.1.2 Total fresh and dry weights (g) per plant.

Concerning the effect of nitrogen and manganese foliar spray on total fresh weight per plant obtained from the two cuts every season, data in Table (1) indicate that ammonium sulfate with (1000 ppm and 2000 ppm) caused a significant increase of total fresh weight of damsisa herb per plant which were (567.58 g. and 635.05 g) in the first season and (576.10 g and 620.95 g) in the second season, respectively. Meanwhile, the maximum total fresh weight of damsisa herb per plant reached 741.66 g and 708.14g obtained from using 2000 ppm. ammonium sulfate plus 100 ppm manganese sulfate in the first and second seasons, respectively compared with all treatments.

Fable (1): Effect of ammonium and manganese sulfate as foliar spray on the vegetative growth and yield of Ambrosia maritima L.during 2002 - 2003.

F-12-12			900		Fir	First season			The state of the s	***************************************
		1"Cut			2"Cut		174		-	
Treatments	Plant height cm.	Plant fresh weight	Plant dry w. g.	Plant height cm.	Plant fresh weight	Plant dry weight	fresh W./ plant g.	Total dry W./ plant g.	fresh W./ fecl. kg.	Total dry W./ fed kg.
Control	64.90	224.36	51.22	72.33	305.21	75.85	529.57	127.07	7060.75	1694.22
1000 ppm N	82.00	241.62	56.30	89.00	327.30	84.12	567.58	140.42	7585.41	1872.22
1000 ppm N + 50 ppm Mn	89.47	252.77	60.61	92,23	324.05	83.81	576.82	144.46	7690.74	1926.09
1000 ppm N +100 ppm Mn	93.70	278.34	68.62	92.57	335.36	86.80	613.71	155.42	8182.46	2072.22
2000 ppm N	92.10	298.24	73.93	94.77	336.81	87.18	635.05	161.11	8467.12	2148.08
2000 ppm N + 50 ppm Mn	100.80	31538	80.03	08.66	371.43	96.53	686.82	176.56	9157.24	2354.07
2000 ppm N -+ 100 ppm Mn	104.50	337.72	87.30	104.67	403.94	105.09	741.66	192.39	9888.55	2565.14
L.S.D.	3.06	11.51	3.85	4.96	20.58	6.28	26.10	8.26	348.04	110 13

				š	Second season	on				
1	0000	59 150	96 15	71.23	235.93 53.53	53.53	467.58	105.49	6234.24	1406, 50
Control 1000 ppm	86.40	2.81.98	78.38	86.90	294.12	73.55	576.10	151.93	7681.14	2025 ,68
000 ppm	87.00	291.77	71.86	91.00	306.42	77.95	564.86	149.81	7975.67	1997 .42
1000 prm N +100	90.57	293.21	71.70	91.97	315.38	81.49	60859	153.19	8114.33	204.2.48
2000 ppm	90.10	306.83	78.40	89.70	314.13	80.42	620.95	158.82	8279.26	21 17.55
2000 ppm N + 50	100.80	331.71	86.20	101.00		88.24	672.19	174.44	8962.31	
2000 ppm N + 100	103.33	349.06	89.83	103.90	359.09	94.22	708.14	184.05	9441.70	
SD	3.58	68.8	11.33	11.33 3.35	8.87	3.47	39.49	3.42	145.21	45.58

The same trend was observed on total dry weight of damsisa herb per plant which were (140.42 g and 161.11 g) in the first season obtained from using (1000 ppm and 2000 ppm) ammonium sulfate, respectively compared with 127.07 g in control treatment, and (151.93 g and 158.82 g) in the second season obtained from using 1000 ppm. and 2000 ppm ammonium sulfate, respectively in comparison with 105.42 g in the control.

The maximum increases of total dry weight per plant in the first and second seasons were 192.39 g and 184.05 g, respectively obtained from spraying with 2000 ppm ammonium sulfate plus 100 ppm manganese sulfate.

The increases of total fresh and dry weights of damsisa herb per plant in both the two seasons may be due to the effect of ammonium sulfate as physiologically acid fertilizer had a beneficial effect on manganese uptake by plants which play a role in enzyme activity and or enhanced photosynthesis and indol acetic acid synthesis (Kühn, 1962).

3.1.3. Fresh and dry herb yield per feddan (Kg)

Data presented in Table (1) show that there are a significant increase of the estimated fresh herb yield per feddan (Kg) with the increase of nitrogen and manganese foliar spray in all the treatments. The maximum fresh herb yields per feddan were 9888.55 Kg in the first season and 9441.76 Kg in the second season obtained from the treatment 2000 ppm ammonium sulfate plus 100 ppm manganese sulfate compared with all treatments and control.

Regarding the dry herb yield per feddan data in Table (1) show the same trend as fresh herb yield per feddan. Statistical analysis showed that all foliar fertilization treatments increased the dried herb yield per feddan with highly significant difference compared with the control.

The highest dried herb yield produced from applying 2000 ppm ammonium sulfate plus 100 ppm manganese sulfate treatment reached 2565.14 Kg. and 2453.94 Kg in the first and second seasons, respectively. While the lowest yields were 1694.22 Kg in the first season and 1406.50 Kg in the second season.

3.2 Effect of nitrogen and manganese foliar spray on damsin and ambrosin percentage in *Ambrosia maritima* L. dried herb during the two seasons of (2002 and 2003)

Data presented in Table (2) show that all foliar spraying with ammonium and manganese sulfate treatments increased damsin percentage in the two seasons.

The highest damsin percentage in the first and second seasons was 1.60% produced from spraying with 2000 ppm ammonium sulfate plus 100 ppm, manganese sulfate, compared with the control which gave 0.97% and 0.91% in the first and second seasons, respectively.

The same trend was observed in ambrosin percentages which were 1.60% and 1.55% resulted from 2000 ppm ammonium sulfate plus 100 ppm, manganese sulfate compared with 0.81% and 0.77% in both the two seasons, respectively.

These results are in good agreement with those of Refaat and Balbaa, (2001) on lemon grass plants, who mentioned that, the application of manganese in foliar spray had directed the biosynthesis process towards the formation of some hydrocarbon compounds.

3.2.1 Hypoglycemic effect of damsisa extract on rats

Streptozotocin (STZ) is synthesized by *Streptomycetes* achromogenes and is used to induce both insulin – dependent and non insulin–dependent diabetes mellitus. Injection STZ induced toxic events in β–cells rat pancreas (Szkudelski, 2001).

Present in (Table 3) that the tested extract possesses a significant hypoglycemic effect on blood glucose. Addition of damsisa extract significantly decreased serum glucose in all of the streptozotcin diabetic rats tested. For the fourth group, the mean glucose levels in the rats on 2, 4, 6, 8 and 10 days after the treatment were 370, 355, 270, 240 and 195 mg/dL, respectively. For the fifth group, the serum glucose levels measured on the same assay dates as the fourth group were 350, 318, 225, 210 and 160 mg/dL, respectively. The hypoglycemic effect of the damsisa extract was not observed in normal rats.

The hypoglycemic effects may be exerted through the inhibition of glucose absorption, increase sensitivity of receptors to insulin, insulinase inhibiting effect, stimulation of β -cells of pancreas to secret insulin or stimulation of peripheral tissues uptake of glucose.

Table (2): Effect of nitrogen and manganese foliar spray on damsin and ambrosin percentages in Ambrosia maritima L. air dried herb during the two seasons of (2002 and 2003).

Treatmente	Dai	Damsin %	Ambrosin %	% uiso
1 Cautions	1st Season	2"d Season	1" Season	2"d Season
Control	0.97	0.91	0.81	0.77
000 ppm N	1.42	1.39	1.10	1.10
1000 ppm N + 50 ppm Mn	1.48	1.47	1.59	1.55
000 ppm N +100 pm Mn	1.66	1.61	1.60	1.55
000 ppm N	1.53	1.49	1.17	1.15
2000 ppm N + 50 ppm Mn	1.64	1.58	1.59	1.46
2000 ppm N + 100 ppm Mn	1.69	1.69	1.60	1.55

The same of the sa	
After 6 days After 8 days from treatment	After 10 days from treatment
1	789 ± 6.43
77.0 + 7.00	
V8 5 + 0 62	674 ± 499
100.7 ± 2.00	
1 410 10 + 20 81	18 07 + 90 007
408.11 ± 36.11 410.10 ± 37.01	27.701
81 CC + OFC *	* 105 + 17 69
* 2/0±21.0/	
17 91 - 010 ×	* 160 + 14 67
* 223 ± 20.00	
S duom = C	= cerum
31	G. = group. S = serum

Table (4): Effect of ethanolic extract of damsisa on serum levels of selected compounds in diabetic rats.

	s, transa	s. transaminases	s. AIK.		
experimental diess	s. ALT	s. AST	phosphatase	s. Uric acid	s. Orea
The first G.	U/dL	U/dL	KU/dL	mg / dL	mg / dL
fed on basic diet	59.81 ± 4.61	146.76 ± 13.10	38.14 ± 2.86	29.34 ± 2.11	12.17 ± 1.01
The second G: fed on basic diet +	61.21 ± 5.21	152.14 ± 14.64	37.62 ± 2.67	30.67 ± 2.43	11.89 ± 1.11
given 1 ml damsisa					
The third G:	105.2 ± 9.67	271.15 ± 21.18	24.82 ± 2.1	51.16 ± 4.98	19.21 ± 1.82
The fourth G:	60.61 ± 4.86	148.15 ± 12.11	336.82 ± 2.89	29.88 ± 2.14	14.82 ± 1.11
giver, 1ml damsisa					
The fifth G:	61.21 ± 5.14	150.12 ± 11.21	37.15 ± 2.76	30.11 ± 2.81	15.99 ± 1.19
given 2 ml damsisa extract.					

G. = group. S = serum. Each value represents the mean of 8 rats ± S.E.
P: Values v/ere calculated (t) test, were < 0.01 for all values

Normal value of serum urea in rats: 8.00 - 27.5 mg / 100 ml (Burns and Lannoy, 1966).

The amount of alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase in the diabetic rats are shown in Table (4). In STZ diabetic rats, serum ALT and AST levels were significantly higher than the normal values Table (4). The gluconeogenetic action of ALT and AST could represent a compensatory response by providing new supplies of glucose precursors.

In our study, the treatment of STZ diabetic rats with the damsisa extract led to lowering of serum ALT and AST levels. The high levels of transaminase enzymes found in this study may be due to hepatotoxic effect of STZ. (Szkudelski, 2001). Our STZ – diabetic rats also showed low serum alkaline phosphatase levels as compared to normal control, but the value was increased after the treatment. These results are in agreement with those obtained by Eskander *et al.*, (1995). In this study the elevated urea level in diabetic rats is likely to be due to increased amino acid catabolism, impaired kidney function or liver damage (Lietz and Finley, 1983). It is concluded that the increase in uric acid is associated with diabetic rats (Lietz and Finley, 1983). Under the effect of ethanolic extract of damsisa 2 ml / 100g b.w. insignificantly decreased serum uric acid and urea than the low level (1 ml / 100 g.b.w.) Table (4).

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تأثير الرش بالنيتروجين والمنجنين على النمو والمحصول والمكونات الفعالة لنبات الدمسيسة وتأثير المستخلص العشبي على خفض مستوى السكر في القران المصلية بالسكر

زهيرة توفيق زكى إبراهيم ، عسرواحد مصطفى ، الهام محمد عطية '

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ملتص

أجريت هذه الدراسة في محطة يحوث مريوط في موسمي ٢٠٠٣- ٢٠٠٣ على نبات الدمسيسة لدراسة تأثير الرش يتركيزين ١٠٠٠ و ٢٠٠٠ جسزء فسى المليون من سلفات النشادر مع إضافة ثلاث مستويات من تركيز المنجنيز وهسمى صفر و ٥٠ و ١٠٠٠ جزء في المليون لأي من التركيزين المذكورين.

أظهرت الدراسة زيادة معنوية في طول النبات والوزن الطازج والجساف للعشب لكل نبات وكذلك محصول القدان من العشب، وأوضحت الدراسسة زيادة النسبة المئوية للمواد الفعالة من الدامسين والأمبروزين كنتيجة لاستخدام ٢٠٠٠ جزء في المليون من سلفات النشائر بالاضافة الى ١٠٠ جزء فــــــي المليون من سلفات المنجنيز،

كما أظهرت الدراسة تأثيرا معنويا كبيرا للمستخلص المائي والكحولي لنبات الدمسيسة على مستوى جلوكور الدم في الفئران المصابة بمرض السكر وذلك بعد عشرة أيام من تعاطيها المستخلص وقد استعادت الحيوانات المصابسة النسبة الطبيعية للجلوكور والانزيمات الناقلة لمجموعة الأمين (اسبرتيك ترانسس امينسيز والانين ترانس امينيز) والفوسفاتيز القاعدي وأيضا اليوريا وحمض اليوريك بعسد توقف المعاملات، ووجد أن التغيرات الملحوظة في مستوى الجلوكور والإنزيمات في سيرم الدم تثبت أن مستخلصات الدمسيسة يمكن أن تمثل علاجا فعالا للفستران التي أصيبت بمرض السكر،

المجلة العلمية لكلية الزراعة - جامعة القاهرة - المجلد (٥٥) العدد الرابــــــع (١٠٥) العدد الرابـــــع (اكتوبر ٢٠٠٤): ٥٩ - ٥٨٦-٥١٩.