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A MULTISPECIES HERBICIDE SCREENING TEST FOR THE PHYTOTOXICITY EVALUATION ON SEVEN SPICES.

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ABSTRACT

A herbicide screening experiment determined the phytotoxicity (phyto) effect of norflurazon (NOR), simazine (SIM), bentazon (BEN), dicamba (DIC) and a nontreated control on sage (***Salvia Officinalis*** L.), pot marjoram (***Origanum onites***), wild marjoram (***Origanum vulgare subsp. vulgare***), lavender (***Lavandula angustifolia***), winter savory (***Satureja Montana***), rosemary (***Rosmarinus officinalis*** L.), and sweet marjoram (***Origanum mejorana***). A strip-split plot design with four reps was sprayed with each herbicide at three rates. Phyto rating was determined 21 days after application. Differences between herbicides, rates and spices was significant. Interactions between each pair of factors and between the three treatments were significant. NOR proved to be the least phytotoxic herbicide. NOR was safe at ½ X in wild marjoram, lavender, winter savory, rosemary and sweet marjoram and at 1 X in lavender and rosemary; BEN at ½ X in sage, pot marjoram, winter savory and rosemary, and at 1 X in rosemary; and DIC at ½ X in sage, rosemary and sweet marjoram showed potential. SIM killed the exposed plants at all rates.

INTRODUCTION

The ability to compete in price and quality with traditional herb production areas of the world requires the understanding of production constraints and, the incorporation of appropriate, improved and efficient production technologies (Lewis, 1984), According with

Morris and Craker (1990) an important factor in herb production in the USA is the organic farming philosophy tightly associated with the production of herbs in gardens and small production areas. However, as Ikerd (1989) emphasizes efficient and environmentally sound techniques either of organic or inorganic precedence should be the goal of every production package when more extensive production areas are to be considered.

Hill and Barclay (1987) state that weed impact, control and herbicide effect should be evaluated for any plant been domesticated as a crop. Because of the diverse nature and market value of spices in the global food market, few manufacturers seek to obtain approval for the use of their chemicals on such crops. In the USA hand-weeding is a limiting factor due to high cost of labor. According to Freed and Davies (1980) in many areas of the Caribbean control over the use of pesticides is low, farmers generally pay no attention to the human risk when using pesticides. Moreover, as pointed by Weir and Shapiro (1981) residue levels of pesticides in imported minor crops has become an issue for American consumers. In the above situations, it is important to have labeled herbicides in herbs and spices.

There are a number of herbicides which have been tested in different countries interested in supplying the world herb market. Countries such as England, Israel, France, Russia and Yugoslavia are developing strong herb industries based on modern production practices. The Ministry of Agriculture, Fisheries, and Food (1980) of England had been testing and recommending the following herbicides for use in herbs; aminothiazole, chlorbufam, dalapon, EPTC, glyphosate, paraquat, pentanochlor, simazine, 2,3,6-TBA and trifluralin. Simazine and Terbacil has been labeled as preemergence herbicides in established mint (*Menta* spp), both spearmint and peppermint, at rates of 2 lb and 1.4 lb of commercial product acre^{-1}, respectively (Appleby and Brewster, 1980) and (Daniel,1976). On Sage (***Salvia officinalis*** L.), simazine is labeled post-planting in two applications, in September and in April, at rates of 1 and 2 lb active ingredient (ai) acre^{-1}, respectively. Also on Sage, Propachlor pre-plant incorporated (PPI) at 4 lb ai acre^{-1} has shown promises for the control of annual weeds.

On established lavender (*Lavendula angustifolia*) for oil production, terbacil at 0.8 lb ai acre^{-1} on light soils, and trifluralin at 1 lb ai acre^{-1} incorporated before planting (Kaspova et al, 1980) (Nagy and Szalay, 1977). On Rosemary (*Rosmarinus officinalis* L.) grown as nursery stock, Simazine, PPI at up to 1 lb ai acre^{-1} has been used. According to the Florida PEST-BANK Pesticide Product Data of February 1991, Treflan® and EH 951® are registered for use in Rosemary and Devrinol® is registered for use in Sweet marjoram, Winter savory, and Basil. In Florida no herbicide has been labeled for use on Sage, Lavender, Wild marjoram and Oregano.

Herbicide labeling and weed control is one of the major production constraints for the potential expansion of the herb industry in Florida and the Caribbean. Under these quidelines the objectives were defined as follows: I) Determine the most promising herbicide for each spice for further evaluation under field conditions, II) Determine the rate of tolerance of each spice to herbicides, and III) Evaluate the phytotoxicity symptoms of four post-emergence logarithmic applied herbicides on seven spices.

MATERIALS AND METHODS

Cuttings of seven spices were rotted in January 1990 on perlite for 21 days. Hotbed temperature during rotting was 28°C. Rotted cuttings were transplanted to multi-cell plant tray in Metromix 350 soil-media. During the winter plants were grown under adequate irrigation and weekly fertilization with Peter's 20-20-20 liquid fertilizer at 1 teaspoon per gallon of water. White flies were controlled with safer insecticidal soap® on a preventing schedule at 75 ml per gallon of water.

On June 1990, the plants were transplanted to white disposable styrofoam cups of 0.30 liter fluid capacity. The soil-media was made of 75 % soil from the green acres (G.A.) agronomy farm, mixed with 25 % peat moss. The soil mixture was used in order to use a representative soil of the region while increasing water retention. The mixed soil-media was sterilized with Bromo-o-gas® (methyl bromide). Irrigation was provided daily and fertilization with peter's 20-20-20 was applied when needed.

At the beginning growth of most spices was slow. By December 1990, plants were growing well and roots penetrated through the cups to the soil. Pruning of some of the species was necessary in order to keep the less aggressive ones under optimum growing conditions. The plants were grown at the experimental site until the moment of treatment application on September 11, 1991.

Because of the number of herbs and herbicides included in this study, an innovation of the multispecies screening design proposed by Aldrich (1951) and Talbert et al (1983) was used. Four herbicides were selected to be tested at three rates. The test was set in a strip-split plot design with four replications. Herbicide treatments were the vertical factor (A). The selection of the herbicides was done base on the possibility each product has to be labelled for use on edible crops. Also, these herbicides have a high LD_{50}. Norflurazon (Zorial®)[4-chloro-5-(methylamino)-2-(∞,∞,∞ -trifluoro-m-tolyl)−3(2H)−pyridazinone] was herbicide one with acute oral LD_{50} of 8,000 mg/kg. Simazine (Princep®) 2-chloro-4,6-bis (athylamino)-S-triazine was herbicide two with acute oral LD_{50} of 5,000 mg/kg. Bentazon (Basagran®) (3-(1-methylethyl)-IH-2, 1,3-benzothiadiazin-4(3H)-one 2,2-dioxide) was herbicide three with acute oral LD_{50} of 2,063 mg/kg. Dicamba (Banvel®) 3,6 dichloro-o-anisic acid was herbicide four with acute oral LD_{50} of 1,028 mg/kg. All of these are being widely researched for use on a variety of fruit and vegetables.

Table 1. Selected herbicides and the calculated IX rate.

Herbicide treatments			Crop[§]	Dosis		
No	Common	Trade		Product per acre	lb ai per gal	lb ai per acre
1-	Norflurazon	Zorial	Soybean	1.25 lb	0.80	1.0
2-	Simazine	Princep	Asparagus	3.00 gt	4.0	3.0
3-	Bentazon	Basagran	Peppermint	3.00 pt	4.0	1.5
4-	Dicamba	Banvel	Asparagus	3.00	4.0	0.038
4-	Control	Water	n/a	0.75 pt		

[§] The recommended rate for a sensitive, edible crop was selected. Norflurazon is used mainly on grain and legumes field crops. All selected rates are recommended for course soils.

Rate treatments were the horizontal factor (B). They consisted of three logarithmic increased rates; half of the recommended rate (½ X), the recommended rate (1 X) in pounds of ai per gallon of commercial product recommended for a vegetables crop, and double the recommended rate (2 X). Within each set there were 5 units for each one of the 4 herbicides and a non-sprayed control (Table l). A single experimental unit (one plant of each spice) is included in each unit. Seven spices were considered the sub-plot factor (C). They were the following: 1) Sage (*Salvia Officinalis* L.), 2) Pot marjoram (*Origanum onites*), 3) Wild marjoram (*Origanum vulgare subsp. vulgare*), 4) Lavender (*Lavandula angustifolia*), 5) Winter savory (*Satureja montana*), 6) Rosemary (*Rosmarinus officinalis* L.), and 7) Sweet marjoram (*Origanum majorana*).

The logarithmic spray system specified by Danielson and Gentner (1966) was implemented. It consisted of an adjustable shield unit and a 2 gallons carbon dioxide (CO^2) powered sprayer (Figure 1). The adjustable shield unit was made of two inches pvc tube frame and two plexiglass shields. Burrill et al. (1976) methodology was used to calibrate the equipment at 20 gallons $acre^{-1}$ and 30 psi nozzle pressure using a 8005 tee jet nozzle. Height was controlled by resting and sliding the boom at top of the plexiglass shields on the pvc frame (Figure 1). The spayed area was isolated by placing each sub-plot within the two plexiglass shields. The speed was controlled by the movement of the boom over the shield. The required amount of ai was calculated following Neal (1976) manual for small dosage of pesticides.

The phytotoxicity evaluation was done on October 2, 21 days after treatment applications. Since the effect(s) of a given herbicide on a particular spice is unknown, four different symptoms were considered (Frans et al, 1986). Foliage burning, stunting, chlorosis, and wilting in a scale of 1 to 5 were used as visual criteria. The rating scale was divided as follows: 1= no symptom (active growing plant), 2= 1-20 % of plant affected, 3= 21-50 % of plant affected, 4= 51-80 % of plant affected, 5= 81-100 % of plant affected.

As recommended by Derr and Appleton (1988), and Eagle (1981) only one person should evaluate the treatments so that the phytotoxi-

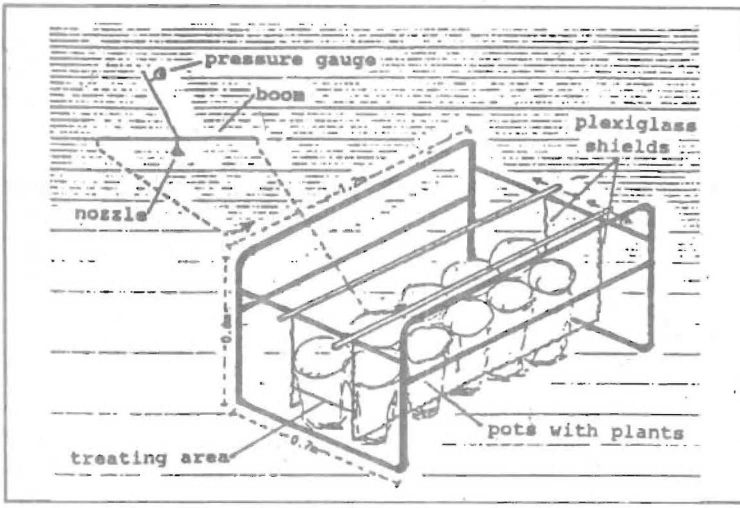

Figure 1. Adjustable shield unit for target and application height control.

city symptoms and intensity are assessed in the same manner. As recommended by Burrill et al (1976) in preliminary screening trials, qualitative data usually satisfy, but in more advanced trials a combination of both qualitative and quantitative data are normally collected.

Statistical analysis included the analysis of variance (ANOVA), least significance difference (LSD) for a strip-split plot design where two main-plot means at the same combination of subplot and sub-subplot treatments, following Gomez and Gomez (1976) procedure. Each mean was compared against each other and against the control. A single LSD value (1.119) was used for comparison of all means, because the mean square of E (A), E (B) and E (C) are not significance larger than E (D) (Table 2). Also, the minimum significance difference (MSD) was calculated. It allowed the selection of those herbicides and/or rates which did not caused phytotoxicity symptoms significantly different from the average of the three controls included at each rate. Data were analyzed using MSTAT 4.0 statistical program.

RESULTS AND DISCUSSION

The analysis of variance showed a highly significant difference between herbicide, rate and spice treatments. Also, there was highly significant interactions between each pair of factors and between the three treatment factor. Also, there was a significant difference for the three two-factor interactions; herbicide-rate (A X B), herbicide-spice (A X C), and rate-spice (B X C) (Table 2). The interactions A X B and A X C were significant at 0.01 level of probability. While, the B X C interaction was significant at the 0.05 level.

Table 2. Analysis of variance of a 3-factor experiment with split treatments arranged in strips.

Source	Degrees of Freedom	Mean Square	F value	Probab.	Level of Significance
Replicati	3	1.988	2.04	0.178	NS
on					
Vertical	3	45.790	47.10	0.000	**
Factor (A)	9	0.972			
Error (A)					
Horizont	2	91.128	387.58	0.000	**
al Factor	6	0.235			
(B)	6	7.906	10.20	0.000	**
Error (B)	18	0.775			
A X B					
Error (C)	6	15.565	24.83	0.000	**
Subplot	18	1.609	2.57	0.000	**
Factor (C)	12	1.368	2.18	0.013	*
A X B	36	1.090	1.74	0.008	**
B X C	216	0.627			
A X B X C	335				
Error (D)					
Total					

Legend: factor(A)= herbicide, factor(B)= rate, and factor(C)=spices. (*,**)= different at the 95% and 99%, respectively. (NS)= nonsignificant.

Least significant difference (LSD) analysis between herbicides over all rates and spices showed norflurazon to be the less toxic herbicide with a rating of 3.0. Bentazon and dicamba followed with an average phytotoxicity rating of 3.56 and 4.03, respectively. The highest phytotoxicity rating was obtained with simazine (4.77) which killed most of the exposed plants when the herbicide rate was doubled. The average for the three rates over all herbicides and spices followed the expected trend. Using half of the recommended rate (½ X) was the least phytotoxic followed by the recommended rate (1 X), and the worst phytotoxic response was obtained by doubling the rate (2 X) which killed most of the plants. Since the data showed high level of interaction, the results and discussion of each spice will be in separate sections.

Sage

Phytotoxicity increased with the herbicides rates. At ½ X Bentazon and Dicamba were not significant different from the control (Table 3). The MSD value (0.914) over the control means (1.25), place these herbicides within the area of acceptance (Figure 2). At 1 X and 2 X, all herbicides caused phytotoxicity greater than 50 % (average rating of 3.0). Norflurazon appears to be the least phytotoxic when used at 1 X and 2 X rates with average rating of 3.50 and 4.00 respectively. However, LSD analysis showed all herbicides were different from the control at 1 X and 2 X rates.

Sage was sensitive to Simazine at all rates, having symptoms of necrosis even at the ½ X rate. Dicamba caused twisted, virus-like leaves and malformation of tips at 1 X. Bentazon did not caused phytotoxicity at ½ X, the foliage was necrotic at 1 X and 2 X rates. Norflurazon at ½ X caused the senescence of some leaves, but the plants recovered.

Pot marjoram

Pot marjoram was the most sensitive to increased rates of herbicides, among the seven spices. All herbicides and rates caused phytotoxic damage to the plants which were significantly different from the untreated controls (Table 4). However, the MSD value (0.914) over the controls mean (1.67), place Bentazon at ½ X within the margin of acceptance for further research (Figure 3). At 1 X and 2 X the four herbicides caused phytotoxicity symptoms greater than 50 %. As in Sage, Norflurazon was the least toxic when used at 1 X and 2 X rates. Simazine and Dicamba were lethal even at ½ X. At the 2 X rate, LSD analysis showed all herbicides different from the control at P = 0.05.

The phytotoxicity symptoms to Pot marjoram were as follows. Norflurazon caused bleaching of the foliage at ½ X and 1 X. Leaves were pale with between vein chlorosis. Bentazon caused the chlorosis of tender leaf tissue at ½ X and the dead of the plant at higher rates. Simazine and Dicamba were very toxic at all rates, causing rapid plant necrosis and dead.

Wild marjoram

A varied degree of phytotoxic symptoms among herbicides were obtained with wild marjoram. At ½ X rate, norflurazon and bentazon were not significance difference (P = 0.05) from the control (Table 3). At 1 X, norflurazon was the only herbicide no significant difference from the control. At 2 X, LSD value found significance difference between all herbicides and the control, most herbicides caused the burning and dead of the plants. MSD value (0.914) over the control mean value allows the use of norflurazon and bentazon in further research (Figure 4). At ½ X, both herbicides were less than 50 % phytotoxic. While at 1 X only norflurazon did not exceed the 50 % damage to the foliage.

Since the level of tolerance of wild marjoram to norflurazon appears to be high, only a yellowing of the tips was observed at ½ X and 1 X. Bentazon stunted the plants, and malformation of the tips was observed at ½ X. Simazine was very toxic to wild marjoram, causing complete necrosis at all rates. Also, dicamba caused necrosis at all rates.

Lavender

This oil producing herb was relatively tolerant to the herbicides under evaluation. At ½ X and 1 X, norflurazon phytotoxicity was not significantly different from the control (P = 0.05) (Table 5). At both rates, norflurazon did not caused phytotoxicity symptoms in 20 % of the foliage. The MSD value (0.914) over the control mean (1.08), allows the selection of norflurazon for further testing (Figure 5). At 2 X all herbicides caused very phytotoxic effects, the killing of all plants in a set was common.

Norflurazon was the least toxic of the herbicides under study. At ½ X not phytotoxic symptom was apparent, but at 1 X some chlorosis could be seen. Bentazon caused burning of the foliage. Dicamba caused yellowing of the tips at low rate and dead of plants at 2 X. Simazine was very toxic at all rates causing rapid necrosis and dead of the plants.

Winter savory

Winter savory was a difficult plant to growth under the conditions of the experiment. Before spray the plants were not completely healthy which produce a high control mean (3.08). However, a trend existed in the data. At ½ X norflurazon and bentazon were not significance different from the control. Also, at 1 X bentazon was not significance different from the control (Table 3). However, the bad conditions of the control plants gave a high MSD value (4.02) which allows the selection for further research of norflurazon, bentazon and dicamba at ½ X and norflurazon and bentazon at 1 X (Figure 6). Bentazon at ½ X did not caused phytotoxicity symptoms in more than 50 % of the foliage. At 2 X all herbicides killed the plants.

Table 3. Least significant difference (LSD) comparison between herbicide means at three rates and LSD between herbicide means and their control.

Herbicide	Rate ½ X	Rate 1 X	Rate 2 X
Sage			
Norflurazon	2.25 a b	3.50 a	4.00 a
Simazine	3.75 a	5.00 a	5.00 a
Bentazon	1.75 b	4.00 a	4.75 a
Dicamba	2.00 b	4.25 a	5.00 a
Control	1.00 b	1.50 b	1.25 b
Pot marjoram			
Norflurazon	2.75 b	3.50 b	5.00 a
Simazine	5.00 a	5.00 a	5.00 a
Bentazon	2.50 b	5.00 a	5.00 a
Dicamba	5.00 a	5.00 a	5.00 a
Control	1.00 c	1.25 c	2.75 b
Wild marjoram			
Norflurazon	1.25 b	2.00 b	4.50 a
Simazine	5.00 a	5.00 a	5.00 a
Bentazon	2.00 b	4.25 a	5.00 a
Dicamba	4.25 a	5.00 a	5.00 a
Control	1.00 b	1.50 b	1.50 b
Lavender			
Norflurazon	1.25 b	1.75 c	4.75 a
Simazine	4.50 a	5.00 a	5.00 a
Bentazon	2.50 b	3.75 b	5.00 a
Dicamba	2.25 b	3.75 b	5.00 a
Control	1.00 b	1.25 c	1.00 b
Winter savory			
Norflurazon	3.25 b	4.00 a	5.00 a
Simazine	5.00 a	5.00 a	5.00 a
Bentazon	3.00 b	3.75 a	5.00 a
Dicamba	3.75 b	4.50 a	5.00 a
Control	2.25 b	2.75 a	4.25 a
Rosemary			
Norflurazon	1.00 b	1.50 b	3.50 a
Simazine	3.50 a	4.75 a	4.25 a
Bentazon	1.25 b	1.75 b	4.00 a
Dicamba	1.25 b	2.25 b	3.75 a
Control.	1.25 b	1.25 b	1.25 b
Sweet marjoram			
Norflurazon	1.75 b	3.00 b	4.25 a
Simazine	4.50 a	5.00 a	5.00 a
Bentazon	3.50 a	4.25 a	5.00 a
Dicamba	3.25 a	4.50 a	5.00 a
Control	2.00 b	3.00 b	2.25 b

note: mean followed by the same letter (a, b, c, d) are nonsignificant difference between them at P=0.05.

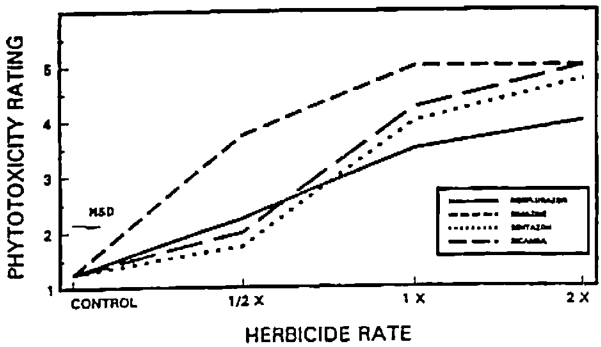

Figure 2. Response of Sage to increasing rates of four selected herbicides

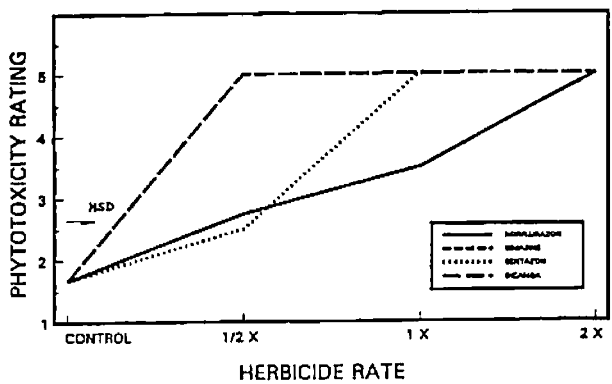

Figure 3. Response of Pot marjoram to increasing rates of four selected herbicides

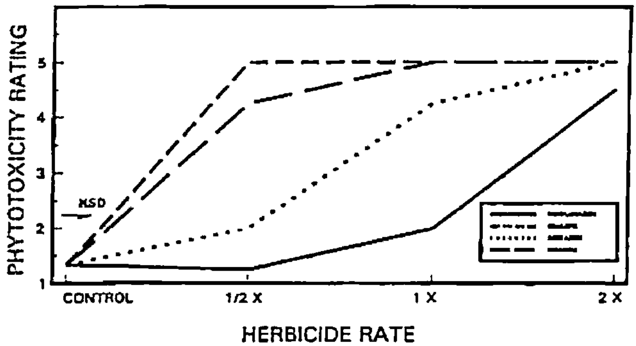

Figure 4. response of Wild marjoram to increasing rates of four selected herbicides

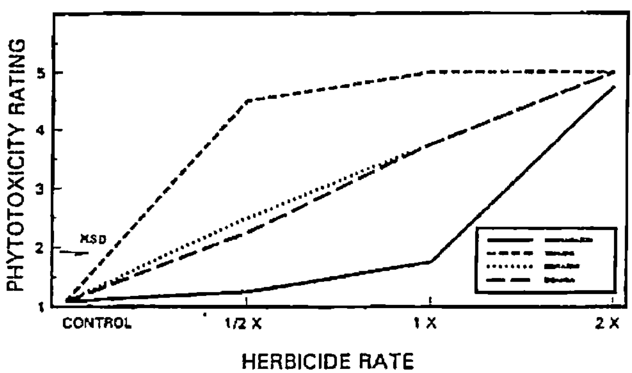

Figure 5. response of lavender to increasing rates of four selected herbicides

The phytotoxicity symptoms on winter savory can be described as follows. Norflurazon at ½ X caused the yellowing and some necrosis of the leaf tips across plant canopy. Simazine caused the complete necrosis and death of the foliage at all rates. Bentazon at ½ X did not demonstrated phytotoxicity symptoms, but at 1 X some bronzing was observed. Dicamba caused bleached, virus-like malformations of the tips.

Rosemary

Rosemary was the spice most tolerance to increased rates of herbicides. At ½ X and 1 X rates norflurazon, bentazon and dicamba caused phytotoxicity damage not significant difference from the control (Table 3). The only exception was simazine which at all rates caused phytotoxicity symptoms greater than 50 %. The MSD value (0.914) over the control means (1.25) allows the use of norflurazon, bentazon and dicamba at ½ X in further research. Norflurazon and bentazon can be used at the 1 X rate (Figure 7).

Minor phytotoxicity symptoms in Rosemary were the following. Norflurazon at 2 X caused chlorosis of the foliage, but plants recovered 1 month after spraying. Simazine, the most toxic of the herbicides, caused severe burning and necrosis to more than 80 % of the foliage at all rates. Bentazon and Dicamba caused necrosis and dead of the plants at 2 X.

Sweet marjoram

Sweet marjoram was a difficult plant to growth. At herbicide application the plants were not completely healthy giving a control mean of 2.42. At ½ X and 1 X norflurazon was not significance difference from the control. At 2 X all herbicides were significance difference from the control (Table 3). The MSD (0.914) value over the control means (2.42) permits the use of norflurazon and dicamba at ½ X in further research. Also, norflurazon can be used at the 1 X rate (Figure 8).

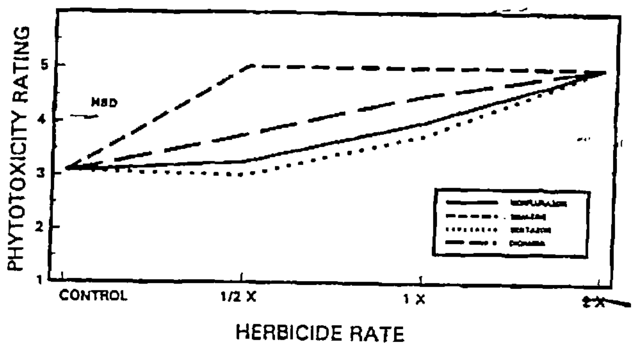

Figure 6. response of Winter savory to increasing rates of four selected herbicides

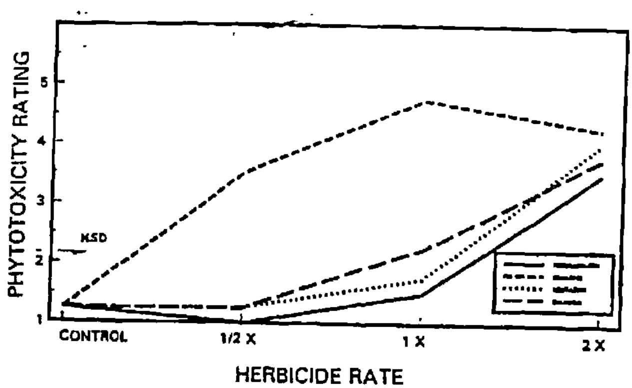

Figure 7. response of Rosemary to increasing rates of four selected herbicides

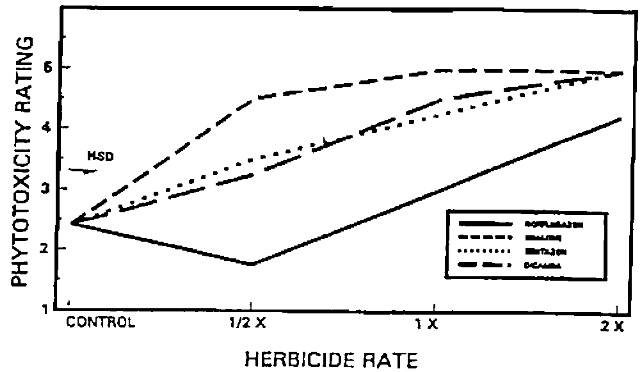

Figure 8. Response of Sweet marjoram to increasing rates of four selected herbicides

The phytotoxic symptoms observed on Sweet marjoram were as follows: norflurazon at 1 X and 2 X caused loss of foliage of the plants, bentazon and dicamba at all rates caused yellowing and defoliation in various degrees, simazine was very toxic causing the dead of plants at all rates.

CONCLUSIONS

The screened herbicides, produce different phytotoxicity levels on the seven spices under study. Phytotoxicity increased with rate increase. On the average, over all spices, norflurazon was the least toxic herbicide. It was followed by bentazon and dicamba. The worst phytotoxicity rating was obtained with simazine which caused necrosis and senescence of sprayed plants at 1 X and 2 X rates.

The MSD value allowed the further testing of the herbicides which did not caused phytotoxicity damage which differentiate from the control plants. On Sage, a relative tolerance spice, bentazon and dicamba at ½ X should be tested under field conditions. They caused some leave senescence, but the plants recovered. On Pot marjoram, the most sensitive of all spices, bentazon at ½ X gave promising results. However, chlorosis of tender leaves was observed. On Wild marjoram, a relatively tolerance spice, norflurazon and bentazon at ½ X gave promising results for further research. Although, some yellowing of the tips and stunting of plants were produced by both herbicides, respectively.

On Lavender, a tolerance spice, norflurazon at 1 X should be further researched. No phytotoxicity was observed at this rate. On Winter savory, a spice difficult to growth, norflurazon and bentazon at ½ X are promising herbicides. Some yellowing of the tips were caused by norflurazon. No phytotoxicity was produced by bentazon at this rate. On Rosemary, the most tolerant spice, norflurazon, bentazon and dicamba at ½ X and norflurazon and bentazon at 1 X can be tested under field conditions. No phytotoxicity symptoms were observed at both rates. On Sweet marjoram, a spice difficult to growth under the experimental conditions, norflurazon and dicamba at ½ X can be further tested under field conditions. Both herbicides caused yellowing and the lost of the foliage at various degrees. The phytotoxicity symptoms evaluated for each one of the spices agreed with consulted literature.

The use of replicated herbicide screening tests is of great utility in obtaining preliminary herbicide data. Herbicide phytotoxicity on new crops and cultivars can be obtained for several herbicides and rates with accuracy. The most promising products can be selected before extensive and time consuming field experiments are needed. Weed control, residue analysis and tolerance levels should be determine before these products can be label on the tested spices.

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