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THE INFLUENCE OF ADDING MORPHACTIN TO THE HERBICIDAL ACTION OF BROMOXYNIL ON THE GROWTH, CHEMICAL COMPOSITION AND ANATOMICAL STRUCTURE OF CHARD PLANT

Saad El-Din, Samia A.; Sonaa H. El-Gayer and Sanaa A. Mohamed
Botany Department, National Research Centre, Dokki, Cairo, Egypt

ABSTRACT

Two pot experiments were carried out during the two successive seasons of 2001/2002 and 2002/2003 in the green house of the National Research Centre, Dokki to investigate the effect of spraying Bromoxynil at the concentrations of 0, 1.00, 1.25 and 1.50 mg/l and Morphactin at concentration of 0, 20, 40 and 80 ppm alone or in combination on the growth after 30 and 60 days from spraying *Beta vulgaris*, L. var Folliosa A. SF. Leaf beet (Chard). Chemical composition was determined after 60 days from spraying.

The anatomical studies were performed in the leaves of three months old plants treated with the lower concentration of Bromoxynil (1 mg/l) or Morphactin (20 ppm) as well as plants treated with the combination between (Morphactin 40 ppm + Bromoxynil 1.00 mg/l), since the high concentration of both substances induced complete eradication of Chard.

Combined treatment 40 ppm Morphactin and 1.00 mg/l Bromoxynil increased thickness of midvein, but decreased number of vascular bundles. While, thickness of the leaf at its marginal portion was not affected. At the same time, such treatment decreased thickness of the upper and lower epidermis. While, palisade tissue thickness was increased. However, spongy tissue was not affected.

Morphactin and Bromoxynil induced also decreases in N, P and K with the increase in concentration. While, Zn and Mn were increased with the increase in concentration of both Morphactin and Bromoxynil.

These results showed clearly that adding low concentration of Morphactin increased the efficiency of the herbicidal properties of Bromoxynil.

INTRODUCTION

It has been reported by several workers that Bromoxynil is very effective in controlling many broad leaved weeds in many crops (El-Desoki, 1990, Ahmed *et al.*, 1993, El-Desoki *et al.*, 1993, Sultan *et al.*, 1995, Tredaway *et al.*, 1997, Attalla *et al.*, 1998, Nagla, 1998, Sultan *et al.*, 1999 and Reddy, 2001). However, the efficacy of Bromoxynil application depends on the weed species, plant age and rate of application (Oliver *et al.*, 1990; Scott *et al.*, 1996; Roy and Zambaux, 1996; Guerin, 1996 and Prostko *et al.* (1998).

Morphactins are a group of synthetic plant growth regulators with a high potency, which affect almost every phase of plant growth and development (Sunddberg *et al.*, 1994). Morphactin causes inhibition or retardation of a large spectrum of herbaceous and woody species and control the broadleaf weed species and seedling grass (Jaiswal and Bhambie, 1985). It penetrates readily into herbaceous plants (via leaves and/or roots). The compound moves freely inside the plant (acro and basipetal transport).

Accumulation is mainly in growing tips of shoots, roots and also in buds. The growth and development of growing tips and buds of herbaceous plants are blocked or slowed down (Ali *et al.*, 2003).

Under field condition it has been reported by Smith (1998) that some weeds were Bromoxynil-resistant such as *Senecio vulgaris* and the rate required to reduce their growth by 50% was 10 times higher for the resistant biotype than for susceptible biotype.

The aim of this work is an attempt to increase the efficiency of the herbicidal properties of Bromoxynil by adding low concentration of Morphactin on plants of *Beta vulgaris*, L. var Folliosa A. SF. (leaf beet, chard).

MATERIALS AND METHODS

Two pot experiments were carried out at green house of the National Research Centre, during two successive seasons of 2001/2002 and 2002/2003. Chard seeds were sown in pots 50 cm diameter filled with Nile suspended matter and sand, kept outdoors and fed regularly with NPK fertilizers (4:3:2). Ten seeds were planted in each pot. Plants were thinned to six plants/pot after 15 days from sowing. Chard seeds were obtained from Agricultural Research Center of the Ministry of Agriculture.

The experiment included sixteen weed control treatments in four replicates arranged in complete randomized design. Weed control treatments were as follows:

1. Unweeded (control)
2. Bromoxynil 24% Ec (3, 5 – dibromo-4-hydroxy benzonitrile) or Buctrril and Brominal at a rate of 1 mg/l.
3. Bromoxynil at a rate of 1.25 mg/l
4. Bromoxynil at a rate of 1.50 mg/l

Morphactin CF_{125} (2-chloro-9- hydroxyfluorene -9- carboxylic acid) supplied by E-Merck Co., was foliar sprayed to sea beet at the concentrations of:

5. Morphactin at a rate of 20 ppm
6. Morphactin at a rate of 40 ppm
7. Morphactin at a rate of 80 ppm
8. Bromoxynil at 1 mg/l + Morphactin at a rate of 20 ppm
9. Bromoxynil at 1 mg/l + Morphactin at a rate of 40 ppm
10. Bromoxynil at 1 mg/l + Morphactin at a rate of 80 ppm
11. Bromoxynil at 1.25 mg/l + Morphactin at a rate of 20 ppm
12. Bromoxynil at 1.25 mg/l + Morphactin at a rate of 40 ppm
13. Bromoxynil at 1.25 mg/l + Morphactin at a rate of 80 ppm
14. Bromoxynil at 1.50 mg/l + Morphactin at a rate of 20 ppm
15. Bromoxynil at 1.50 mg/l + Morphactin at a rate of 40 ppm
16. Bromoxynil at 1.80 mg/l + Morphactin at a rate of 80 ppm

Chard plants were sprayed with either Bromoxynil alone or Morphactin and Bromoxynil + Morphactin together, their mixture after 30 days from sowing.

Data recorded:

A. Vegetative growth parameters of Chard (Mean of three plants from each pot after 30 and 60 days from spraying).

1. Length of longer leaf (cm)
2. Number of leaves/plant
3. Fresh weight of vegetative growth (g).
4. Dry weight of vegetative growth (g).

B. Chemical composition of plant leaves:

Leaves at the age of three months (after two months from treatments) were dried at 70°C for constant weight to determine macro and micro nutrient elements (N, P, K, Zn and Mn). Concentrations were determined according to the Official and Modified Methods of analysis (A.O.A.C., 1984).

C. Anatomical studies:

Specimens of Sea beet were taken in the middle of leaves developed on the median portion of the main stem of normal and plants treated with Bromoxynil (1mg/l) or Morphactin (20 ppm) as well as plants treated with the combination between Bromoxynil (1 mg/l) and Morphactin (40 ppm). Plants used for examination were taken at the age of three month i.e. after two months of treatments.

Specimens were killed and fixed for at least 48 hr in F.A.A. (10 ml formalin, 5 ml glacial acetic acid and 85 ml ethyl alcohol 70%). The selected materials were washed in 50% ethyl alcohol, dehydrated in normal butyl alcohol series, embedded in paraffin wax of melting point 56°C, sectioned to a thickness of 20 micron, double stained with safranin-light green, cleared in xylene and mounted in Canada balsam (Willey, 1971). Sections were read to detect histological manifestations of noticeable responses resulted from spraying with Bromoxynil or Morphactin and their combinations compared to the control.

D. Statistical analysis:

All data were statistically analysed using the least significant difference test (L.S.D.) at 5% level of probability (Snedecor and Cochran, 1982).

RESULTS AND DISCUSSION

The results in Tables (1 and 2) show that Bromoxynil at all used concentrations induced significant decreases in the fresh and dry weights of Chard after 30 and 60 days from spraying. Moreover, Morphactin induced significant decreases also with 40 and 80 ppm.

The interaction between the two factors proved insignificant in the two stages of growth. It is clear from the Tables (1 and 2) that applying Bromoxynil with the lowest concentration induced sharp decrease in the fresh and dry weights of Chard, the middle and the higher concentration were more

or less similar. The data show also the synergestic toxic effects between Bromoxynil and Morphactin since the high concentration of both substances induced complete eradication of Chard. The number and length of leaves of Chard after 30 and 60 days from spraying are also recorded in Tables (1 and 2). It is worthy to mention that although increasing the concentration of both Bromoxynil or Morphactin induced gradual and significant decrease in the number and length of leaves of Chard except the low concentration of Morphactin increases the number of leaves, yet the interaction between the two factors did not attain the level of significance.

The efficiency of Bromoxynil in controlling broad spectrum of annual broad-leaves weed is in agreement with those reported by Sultan *et al.* (1995), Tredaway *et al.* (1997) and Prostko *et al.* (1998). With regard to the effect of Morphactin on the growth and development of Chard our results are also in agreement with those reported by Sharma and Vatsa (1976) and Smolinski (1976); Kackar *et al.* (1978); Murthy and Inamadar (1980); El-Bassiouny (1992) and Sundberg *et al.* (1994).

The data in Table (3) show the effect of both Morphactin and Bromoxynil on N, P, K as well as Zn, Mn content of Chard leaves. Generally, the lower concentration of Morphactin (20 ppm) increased the amount of N, P and K in the leaves of Chard. On the contrary, 40 and 80 ppm of Morphactin decreased the content of N, P and K. The decrease in N content induced by Morphactin is in agreement with those reported by El-Bassiouny (1992). It is also evident from the data that Bromoxynil induced pronounced and gradual decrease in the amount of N, P and K since the decrease is higher with the increase in Bromoxynil concentration.

The effect of both Morphactin and Bromoxynil on the content of Zn and Mn differs from those of the major nutrients. Morphactin at all concentrations used (0, 20, 40 and 80 ppm) was more or less similar, however, Bromoxynil induced pronounced increase with increasing concentration (0, 1.0, 1.25 and 1.50 mg/l).

Anatomical studies:

As inferred earlier throughout the morphological investigations, Bromoxynil at all concentrations and Morphactin with the middle and higher concentrations induced inhibiting effect in all morphological characters. Moreover, plants treated with the combination between Bromoxynil at the lower concentration (1 mg/l) and Morphactin at 40 ppm showed that Morphactin could enhanced the inhibiting effect of Bromoxynil. This may justify a further study on the internal structure of the leaves of plants sprayed with the above mentioned treatments.

Certain microscopical characters of a leaf blade taken from leaves developed on the middle portion of the main stem of Chard as affected by Bromoxynil (1mg/l), Morphactin (20 ppm) and Bromoxynil (1 mg/l) + Morphactin (40 ppm) were followed up in forms of counts and measurements being given in (Table 4). These characters in both of the control and treated plants are further shown as microphotographs illustrated in Figure 1 (A, B, C and D).

Table (1): Effect of Morphactin and Bromoxynil alone or incombination on Chard first sample after 30 days from spraying (combined analysis of two seasons).

Characters	Conc.	Morphactin ppm																			
		Fresh weight of leaves (g)					Dry weight of leaves (g)					No. of leaves					Length of leaves (cm)				
		0	20	40	80	$\bar{X}$	0	20	40	80	$\bar{X}$	0	20	40	80	$\bar{X}$	0	20	40	80	$\bar{X}$
Bromoxynil mg/l	0	16.00	17.60	8.40	5.33	11.83	6.60	6.90	5.70	2.33	5.38	30.00	38.00	19.00	13.00	25.00	40.0	33.0	19.0	13.0	26.25
	1.00	13.80	8.10	6.20	4.00	8.03	5.40	3.60	2.91	1.39	3.33	27.00	33.00	18.20	11.50	22.43	26.0	20.00	15.00	10.10	17.78
	1.25	8.60	5.80	4.20	2.33	5.23	3.10	2.13	1.52	1.00	1.94	22.00	23.00	16.00	10.00	17.75	19.0	16.00	12.00	8.10	13.78
	1.50	5.80	2.90	1.00	---	2.43	2.63	0.82	0.51	---	0.99	8.50	12.00	10.50	---	7.75	16.3	10.30	6.00	---	8.15
	$\bar{X}$	11.05	8.60	4.95	2.92		4.43	3.36	2.66	1.18		21.88	26.50	15.93	8.63		25.33	19.83	13.00	7.80	

LSD 0.05				
Bromoxynil	1.00	0.24	2.30	2.50
Morphactin	0.91	0.12	2.90	3.10
Interaction	0.30	0.05	NS	N.S.

Table (2): Effect of Morphactin and Bromoxynil alone or incombination on Chard second sample after 60 days from spraying (combined analysis of two seasons).

Characters	Conc.	Morphactin ppm																			
		Fresh weight of leaves (g)					Dry weight of leaves (g)					No. of leaves					Length of leaves (cm)				
		0	20	40	80	$\bar{X}$	0	20	40	80	$\bar{X}$	0	20	40	80	$\bar{X}$	0	20	40	80	$\bar{X}$
Bromoxynil mg/la	0	47.20	49.60	29.80	19.30	36.48	16.30	18.80	8.40	4.80	12.08	40.00	45.00	22.00	17.00	31.00	64.00	45.00	29.90	18.80	39.43
	1.00	19.20	17.68	10.60	7.33	13.70	8.00	6.33	4.10	2.30	5.18	29.00	39.00	20.50	15.30	25.95	36.00	32.00	20.00	14.00	25.50
	1.25	12.55	9.33	7.70	4.00	8.40	5.18	3.00	2.01	1.33	2.88	26.30	25.00	19.22	12.50	20.76	26.20	20.10	16.50	10.80	18.40
	1.50	7.60	5.08	2.00	---	3.67	3.50	1.75	1.00	---	1.56	10.00	16.10	13.30	---	9.85	20.00	15.30	9.50	---	11.20
	$\bar{X}$	21.64	20.42	12.53	7.66		8.25	7.47	3.88	2.11		26.33	31.28	18.76	11.20		36.55	28.10	18.98	10.90	

LSD 0.05				
Bromoxynil	6.20	0.81	8.20	7.50
Morphactin	8.90	0.50	3.00	5.30
Interaction	1.30	0.20	N.S.	N.S.

Table (3): Effect of Morphactin and Bromoxynil alone or incombination on the content of N, P and K as well as Zn and Mn in Chard leaves.

Characters	Conc.	Morphactin ppm																			
		NPK content (mg/g)												Micro content (mg/g)							
		N				P				K				Zn				Mn			
		0	20	40	80	0	20	40	80	0	20	40	80	0	20	40	80	0	20	40	80
Bromoxynil mg/l	0	29.0	37.0	16.0	13.0	31.0	39.0	21.0	18.0	33.0	35.0	24.0	21.0	88	88	89	91	39	39.0	40.0	40.0
	1.00	16.0	18.2	12.0	11.0	23.2	26.4	17.7	15.0	20.2	19.0	19.2	16.0	112.0	104.0	113.0	119.0	51.0	50.0	52.0	52.0
	1.25	13.3	13.9	11.2	10.2	18.0	18.9	12.7	10.2	18.0	16.0	12.0	11.3	121.1	117.0	123	127	65.0	63.0	67.0	68.0
	1.50	11.3	9.9	9.3	---	15.0	11.3	8.9	---	13.0	10.9	8.7	---	129.0	129	131	---	72.0	73.0	72.0	---

Table (4): Counts and measurements (in micron) of certain anatomical characters in transverse sections through leaf blade taken from leaves developed on the median portion of the main stem of Chard plant (*Beta vulgaris* var. Folliosa A. SF.) affected by Bromoxynil (1mg/l), Morphactin (20 ppm) and Bromoxynil (1 mg/l) + Morphactin (40 ppm). (Means of three sections from three specimens).

Histological characters	Control	Bromoxynil% ±to (1mg/l) control	Morphactin% ± to (20ppm) control	(1mg/l)Bromoxynil + 40 ppm Morphactin % ± to control
Thickness of midvein	1242.0	747.0 - 39.9	1414.0 + 13.9	1343.0 + 8.1
No.of vascular bundles/midvein	3.0	1.0 - 66.7	4.0 + 33.3	2.0 - 33.3
Vessel diameter	30.3	23.6 - 22.1	32.1 + 5.9	19.2 - 36.6
Thickness of lamina	289.5	491.5 + 69.8	225.6 - 22.1	287.9 - 0.6
Thickness of upper epidermis	26.3	25.3 - 3.8	3.6 - 10.3	20.2 - 23.2
Thickness of lower epidermis	23.6	20.2 - 14.4	18.5 - 21.6	20.2 - 14.4
Thickness of mesophyll	245.8	419.2 + 70.6	191.9 - 21.9	262.6 + 6.8
Thickness of palisade tissue	134.7	237.4 + 76.2	116.0 - 13.9	151.5 + 12.5
Thickness of spongy tissue	111.10	176.8 + 59.1	75.8 - 31.8	111.1 + 0.0

It is realized that spraying plants of Chard with the herbicide Bromoxynil at (1 mg/l) resulted in abnormal leaves where the thickness of midvein was decreased by 39.9% below the control. The midvein of such treatment had one small vascular bundle against three normal vascular bundles in control plants. Moreover, the diameter of vessels was decreased by 22.1% below the control. Such effect may be due to the effect of Morphactin on cambial activity as reported by Murthy and Inamadar (1980) on *Lycopersicon esculentum* who observed an increase in diameter of xylem vessels with lower concentration of Morphactin whereas an decrease in their diameter were presented with the higher concentration. At the same time, the thickness of lamina was increased by 69.8% over the control. The increase of lamina thickness was mainly due to increase in thickness of both palisade and spongy tissue by 76.2 and 59.1% over the control, respectively. This effect was mainly differ from the data obtained by Smolinski (1976) who found an decrease in the epidermis and mesophyll cell of *Vicia faba* leaves treated with Morphactin. Similar effect was also observed on the leaf epidermas and mesophyll of tissues layers of maize plants treated with higher concentration of Morphactin by Ali *et al.* (2003). While, treated plants had thinner epidermis than that of the control. The decrease in thickness of upper and lower epidermis was 3.8 and 14.4% below the control, respectively.

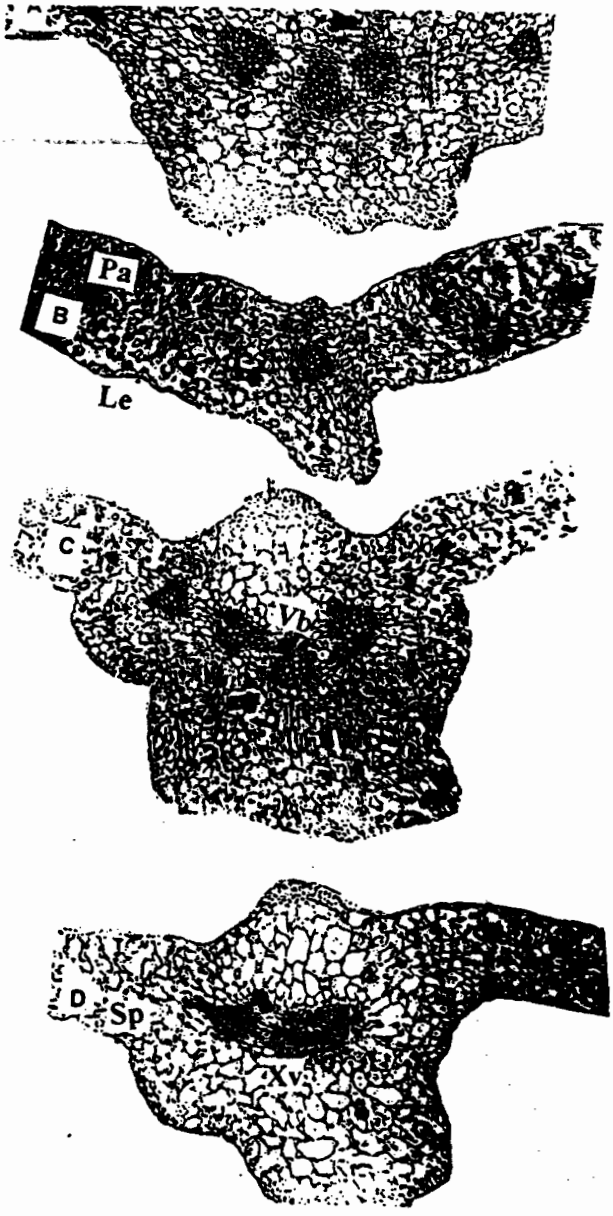

Fig (1): Transverse sections through leaf blade taken from leaves developed on the median portion of the main stem of Chard (*Beta vulgaris*, L. var Folliosa A. SF.) as affected by Bromoxynil and Morphactin (52 X).

A: Untreated plant B: Plant treated with Bromoxynil (1mg/l)
C: Plant treated with Morphactin (20 ppm)
D: Plant treated with Bromoxynil (1 mg/l) + Morphactin (40 ppm)
(Ue): Upper epidermal layer (Le): lower epidermal layer
(Pa) Palisade tissue (Vb) Vascular bundle
(SP) Spongy tissue (XV) Xylem vessles.

As to the effect of Morphactin, it was observed that 20 ppm increased thickness of the leaf at the portion of midvein by 13.9% over the control. The number of vascular bundles was increased by 33.3%over the control. The midvein had four normal vascular bundles against three in control plants. Such effect could be due to the effect of Morphactin on the differentiation and development of leaf vascular tissues as reported by Patel and Setia (1979) and Ali *et al.* (2003). The vessel diameter was increased by 5.9% over the control. On the other hand, lamina thickness was decreased by 22.1% below the control. It is obvious that the thinner lamina induced by Morphactin was mainly due to decrease in thickness of all included tissues (upper and lower epidermis as well as mesophyll tissues). The decreases due to Morphactin effect were 10.3, 21.6, 13.9 and 31.8% below the control for thickness of upper epidermis, lower epidermis, palisade tissue and spongy tissue, respectively. These results are in agreement with those reported in *Vicia faba* by Ali *et al.* (1994).

Concerning the effect of combination treatment between Bromoxynil (1 mg/l) and Morphactin (40 ppm), it was found that such treatment increased thickness of midvein by 8.1% over the control, but decreased number of vascular bundles by 33.3% below the control. While, thickness of the leaf at its marginal portion was not affected. Mean thickness of the leaf lamina was 289.5 and 287.9 microns for control and treated plants, respectively with a negligible difference of 0.6% between them. At the same time, such treatment decreased the upper and lower epidermis by 23.2 and 14.4% below the control, respectively. While, palisade tissue was increased by 12.5% over the control. Such treatment showed no effect on spongy tissue.

These results showed clearly that adding low concentration of Morphactin increased the efficiency of the herbicidal properties of Bromoxynil.

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أثر إضافة المورفاكتين على فاعلية مبيد الحشائش البرومينال على النمو والمحتوى الكيميائى والتركيب التشريحى لنبات السلق

سامية أمين سعد الدين ، صنعة حسين الجيار و سناء عبدالرحمن محمد
قسم النبات – المركز القومى للبحوث – الدقى – القاهرة

أجرى هذا البحث بصوبة المركز القومى للبحوث خلال عامى ٢٠٠١/٢٠٠٢ ، ٢٠٠٢/٢٠٠٣ لدراسة تأثير إضافة كلا من مبيد الحشائش البرومينال بتركيز (صفر ، ١,٠٠ ، ١,٢٥ و ١,٥٠ مجم / لتر) ومنظم النمو المورفاكتين بتركيز (صفر ، ٢٠ ، ٤٠ و ٨٠ جزء فى المليون) إما منفردا أو خلطهما معا على نمو نبات السلق بعد ٣٠ و ٦٠ يوما من الرش ودراسة المحتوى الكيماوى للأوراق بعد ٦٠ يوما من الرش.

أما الدراسة التشريحية فقد أجريت على الأوراق عند عمر ثلاثة أشهر من الزراعة بتركيز ٢٠ جزء فى المليون من المورفاكتين ، ١,٠ ملجم من البرومينال وكذلك الخليط بينهما بتركيز ٤٠ جزء فى المليون من المورفاكتين و ١,٠ مجم/لتر من البرومينال.

أدى استخدام التركيز العالى للمادتين المستخدميتن إلى إبادة كاملة لنبات السلق.

أوضحت الدراسة التشريحية أن تركيز ٤٠ جزء فى المليون من المورفاكتين + ١.٠ مجم/لتر من البرومينال سبب زيادة فى سمك العرق الوسطى للورقة بينما نفس التركيز سبب نقص فى عدد الحزم الوعائية فى حين أن سمك حافة الورقة لم يتأثر.

وفى نفس الوقت لوحظ نقص فى كل من البشرة العليا والسفلى بينما زاد نسيج الطبقة العمادية فى حين لم يتأثر النسيج الأسفنجى.

أظهرت النتائج أن زيادة تركيز المورفاكتين والبرومينال أدى إلى نقص فى كمية العناصر الكبرى (نتروجين ، فوسفور و بوتاسيوم) بينما تركيز العناصر الصغرى (الزنك والمنجنيز) زاد تركيزهما بزيادة تركيز كلا من المادتين المستخدمتين تحت الدراسة.

وتوضح هذه النتائج أن إضافة تركيزات منخفضة من المورفاكتين تزيد من كفاءة مبيد الحشائش البرومينال.