Zagazig J. Agric. Res., Vol. 47 No. (1) 2020 119-133

Plant Protection and Pathology Research

http:/www.journals.zu.edu.eg/journalDisplay.aspx?JournalId=1&queryType=Master

SALTS AS CONTROLLING AGENTS OF LETTUCE LEAF SPOT DISEASES

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Received: 09/10/2019; Accepted: 05/01/2020

ABSTRACT: This study was carried out to evaluate the effect of ammonium sulfate, calcium chloride, potassium dibasic phosphate and sodium carbonate on inhibiting mycelial growth of *Alternaria alternate* (Fr.) Keissler, *Helminthosporium* sp., *Stemphylium botryosum* Wallroth, and *Curvularia lunata* (Wakker) Boedijn isolated from lettuce (*Lactuca sativa* Folium.) leaves. The sodium carbonate and ammonium sulfate strongly inhibited mycelial growth and spore germination. Potassium dibasic phosphate and calcium chloride inhibited mycelial growth to a less extent. Sodium carbonate (25mM) decreased mycelial growth of *A. alternate* by 58.9%, *Helminthosporium* sp. by 58.8%, *S. botryosum* by 78.5% and *C. lunata* by 40.0%. The folpet fungicide used variably decreased growth of fungi in a range varied between 43.5% and 87.5% at 5ppm concentration. The effective concentration (EC50) figures indicated that sodium carbonate and ammonium sulfate caused more inhibition to *A. alternata*, *Helminthosporium* sp., *S. botryosum* and *C. lunata.* Lower concentration of potassium dibasic phosphate and calcium chloride showed lower effect. In general, the most effective chemicals used to control lettuce foliar diseases were sodium carbonate, ammonium sulfate, calcium chloride and potassium dibasic phosphate that decreased leaf spot severity. Sodium carbonate had a higher inhibiting activity against foliar diseases of lettuce*s*. Expression of defense related enzymes involved in lettuce induced tolerance against infection was emphasized. The greater hydrolysis of plant cell walls by *A. alternata, C. lunata, Helmithosporium* sp. and *S. botryosum* was attributed to greater of **p**olyglacturonase (PG) secretion.

Key words: Lettuce, leaf spot, salts, mycelial growth, spore germination, polyglacturonase (PG), *Alternaria alternata* .

INTRODUCTION

Lettuce (*Lactuca sativa* L.) is belongs to the family Asteraceae as a seasonal plant, native to the Mediterranean area and was domesticated in Egypt nearly around 4,500 BC. It is grown where the temperature ranges between 10.6 and 12.7°C during the growing season. It is an important dietary crop available that contains phenolic compounds, carotenoids such as carotene and vitamin E **(Nicollec *et al.*, 2004).** Lettuce is the world's most popular vegetable salad produced commercially in many countries worldwide and exclusively used as a fresh vegetable in salads, but some forms are also cooked (**Raid, 2004, Lebeda *et al.*, 2007**).

Bradley *et al.* (2009) reported that 16 fungal diseases were the most important, prevailing problems on lettuce under greenhouses conditions. The prevention control of fungi is more successful in greenhouse than in open fields.

Foliar spray application of different potassium salts (KCl, KNO_3, K_2SO_4, KH_2PO_4 and K_2HPO_4) have proven to be highly effective inducers of systemic protection against powdery mildew (*Sphaerotheca fuliginea*) in cucumbers **(Reuveni *et al.*, 1995).** Also organic and inorganic salts, on the other hands, have been tried in controlling *Alternaria alternata, Botrytis cinerea, Fusarium. solani* var. *coeruleum, Phytophthora erythroseptica, P. infestans, Verticillium albo-atrum,* and *Verticillium dahlia* (**Mills *et al.*, 2004**). In addition calcium as an essential macronutrient for plant growth and development

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was used **(Hepler, 2005)** and reacts as co-factor of some enzymes in hydrolysis of adenosine triphosphate (ATP) and as second messenger in metabolic and genetic regulation (**Jeter** and **Roux, 2006).**

Sodium is not an essential element but can be used in small quantities, like micronutrients, to aid in metabolism and chlorophyll synthesis and helps in internal water balance. The salts including bicarbonate and carbonate have widely been used in the food industry as preservatives, pH regulators, and antimicrobial agents and are known to have low mammalian toxicity **(Olıvıer *et al.*, 1998)**.

Plants and microorganisms, polyglactronase (PG) are constituted of two types, the endo-PGs (EC 3.2.1.15) and the exo-PGs (EC 3.2.1.67) (**Protsenko *et al*., 2008**). Overall, like PGs from microorganisms such as *Botrytis cinerea*, that show optimal activities at approximately pH 4.5 (**Kars *et al.,* 2005**), plant PGs prefer an acidic pH from 3.3 to 6 **(Verlent *et al.,* 2004**). Polyglactronase activity can also be inhibited by proteinaceous compounds (**Protsenko *et al*., 2008)**.

The objective of the study was to determine the effect of salts on controlling lettuce plants diseases, determine hydrolytic and oxidative activities of polyglacturonase (PG), cellulase (Cx), peroxidase (PO) and polyphenol oxidase (PPO) for the isolated pathogens and to determine the activity of PG and Cx in diseased plant after salts treatments.

MATERIALS AND METHODS

Isolations and Identification of the Pathogens

Isolation was done using lettuce samples collected from Sharkia, Giza and Kalyubia governorates. The diseased lettuce leaves were washed under sterilized water, surface disinfected instantly (1% NaOCl) for 1 min and rinsed several times in sterile distilled water. Excised pieces (4 pieces per plate) were plated on plain water agar. Incubation was made for three days before transferring the developed fungal growth to potato dextrose agar (PDA)medium. Incubation was made at 28°C for 7 days, and stored in refrigerator till use. Purification of the isolated fungi was done using the single spore techniques (**Dhingra and Sinclair (1995).** Identification of the isolated fungi was carried out based on their cultural characteristics according to the identification schemes and microscopic examination (**Neergaard, 1945; Hansford, 1946; Barnett and Hunter, 1972).** Cultures were preserved on PDA medium at 4±1°C till use.

Pathogenicity Test

Hyphal mat of the previously identified isolates were obtained from 7 days old potato broth cultures incubated at 28±2^{O}C. Then the resulted growth mat filtered through Whatman filter pepper and mycelium mat transferred aseptically into 200ml of distilled water solution in warring blender and homogenized for one minute at low speed in order to get the inocula ready for pathogenicity test on lettuce. Spore suspensions (5 ml of 10^5 spore/ml) were prepared in sterilized distilled water of each isolate and determined using Haemcytometer slide according to **Shahin and Shepared (1979**) and atomized onto lettuce leaves, covered with polyethylene bags and incubated at 28±2°C for 4 days in the laboratory under lights . Control lettuce leaves were sprayed with distilled water. Spots were recorded, Koch's postulates were confirmed and determined disease severity by **Biswas *et al.* (1992)**.

Fungal Growth in Liquid Culture

The fungal isolates were cultured and kept on PDA medium (**Dhingra and Sinclair, 1995**) and used to cut discs from the edge of the culture. Discs 5 mm of fungal growth on PDA were transferred to Czapek Dox liquid medium (100 ml) in a 250 ml conical flasks separately and incubated at 28±2°C for 14 days. Three replicates were prepared for each treatment. Whatman No.1 filtration obtained was used to assay polyglacturonase (PG), cellulase (Cx), peroxidase (PO) and polyphenol oxidase (PPO) activities.

Peroxidase assay (PO)

Activity of peroxidase was assayed following the method of **Kar and Mishra (1976).** Five ml of the assay mixture contained (1.5ml of phosphate buffer pH 6.8, 3 ml of 0.05 M pyrogallol solution, and 0.5 ml of 1% H_2O_2) then 1 ml of crude enzyme extract was added.

After incubation for 5 min at 25°C, the color intensity was read spectrophotometerically at 430nm.

Polyphenol oxidase assay (PPO)

Polyphenol oxidase (PPO) was assayed according to the method described by **Kar and Mishra (1976)**. Five ml of the assay mixture contained (3.5 ml of phosphate buffer pH 6.8, 1.5 ml catechol solution) and 1 ml of crude enzyme extract was added. After incubation at 25°C for 5 min. The color intensity was read spectrophotometer at 430nm.

Polyglactoronase assay

Polyglactoronase (PG) activity was determined by measuring decrease in viscosity of the reaction mixtures according to the method of **Mahadevan and Sridhar (1982).** Mixtures containing 2.5 ml of crude enzyme preparations (sample extract), 2.5 ml citrus pectin 1.5% solution, 5 ml in 0.1 M phosphate buffer at pH 6. The reaction mixtures were incubated at 28°C and the loss in viscosity of the mixture using viscometer was measured after 30 minutes against control containing heat inactivated sample extracts instead of the active one.

Cellulase assay

Cellulase (Cx) activity was also assayed viscometrically in mixtures containing 2.5 ml carboxymethyl cellulose 1.5% (CMC) solution, 5 ml 0.1 M phosphate buffer at pH 6 mixed with 2.5 ml crude enzyme preparations (sample extract). The mixtures were incubated at 28°C and the percentage loss in viscosity was estimated after 30 min against control containing heat inactivated sample extracts instead of the active one.

The enzymes (PG) and (Cx) activity were determined in terms of loss of viscosity (%) using the following formula **(Tolbays and Busch, 1970)**.

$$\text{Loss in viscosity (\%)} = T_0 - T_1/T_0 \times 100$$

T_0 = time of flow of blank

T_1 = time of flow treated sample

Mineral Salts and Fungicide Used

Mineral salts and fungicide used are shown in Table 1. All salts were used as 2 and 4 g/l and Folpet fungicide as 1 and 2 g/l. The following treatments were used:

Effect of Mineral Salts on Fungal Growth *in vitro*

Mineral salts solutions were prepared in sterile distilled water and were added to the PDA medium at a desired concentration (5,10,15,20,25,30,35,40 mM). The seven days old cultures were used by taking 0.5 mm diameter disc and placed in the center of each plate. The effect of salts on mycelial growth was assayed according to **Mecteau *et al.*(2002**). The salts concentrations used were covering a range of 5 to 40 mM before autoclaving the PDA medium. Eight concentrations were serially added with 5mM increment in each turn. Fungicide concentrations covering range of 5 ppm to 250 ppm, with 5 ppm increment increase, were added after autoclaving. Control plates with salt- free PDA were used. The plates were incubated at 28±2°C for 7 days. Radial growth was measured and inhibition percentage of growth in relation to controls was calculated using the following:

Inhibition (%) = RG Control- RG Treatment/RG Control × 100

RG=Radial growth

The EC50 concentration of salts

Statistical analysis was used to calculate lower concentration of salts causing 50% decrease (EC50) in mycelial growth of pathogens. Mycelial growth was determined in PDA amended with salts at concentrations, between 5.0 - 40 mM. The data were subjected to statistical analysis by applying (**Bakr, 2007)** software to calculate probity analyses for calculate the regression equation, slope of regression lines, EC_{50} and EC_{90} values of the tested fungicides. The toxicity index (TI) of fungicide was determined according to **Sun (1950)**.

Effect of Salts on Mycelial Morphology and Sporulation

The fungi tested were own kept in PDA medium contain different concentration of tested mineral salts. After seven days incubation, the fungal isolate cultures were subculture to obtain a pure culture. Hyphae of the fungal isolates were examined under a light microscope at (200x) magnification and photographs were taken during observations of hyphal abnormalities.

Table 1. Salts used (Merck chemicals) and Flopet fungicide 80%

Treatment	Chemical formula	Molecular weight g/mol	Concentration used g/l
Ammonium sulphate	$(NH4)_2SO_4$	132.14	(2-4)
Calcium chloride	$CaCl_2$	110.98	(2-4)
Potassium dibasic phosphate	K_2HPO_4	174.2	(2-4)
Sodium carbonate	Na_2CO_3	105.98	(2-4)
Folpet fungicide 80% SC	$C_9H_4C_{l3}NO_2S$	296.6	(1-2)
N-(trichloromethylthio) phthalimide			
2-[(trichloromethyl)thio]-1H-isoindole-1,3 (2H)-dione			

Field Experiment

This study was carried out under natural infection in approximately one Faddan (4200 m^2) area at Inshas, (Sharkia governorate). Seeds of Balady and Dark green lettuce cultivars were obtained from Vegetable Crops Research Dept., ARC, Giza, Egypt. Seeds were sown at depth of 2 cm in clay soil. The field trial, (20 plots) were designed in complete randomized block with three replicates. Each plot was 3x3 m and with four rows of 3m in length and 75 cm in width. The soil irrigated 7 days before sowing date. Lettuce planting and spacing were made at the rate of 2 seedling/hill and 20 cm apart. The efficacy of salt treatments as well as (Folpet 80%) fungicide were determined. The growing plants were sprayed two times at 15 and 45 days as previously mentioned concentrations. Disease severity was assessed according to the scale and formula reported by **Biswas *et al.* (1992)**.

Disease index of symptoms was:

1= (1-2) spots/leaf,

2= (3-5) spots/leaf,

3= (6-10) spots/leaf,

4= up to 25% infected area on the leaf,

5= up to 50% infected area on the leaf,

6= up to 75% infected area on the leaf

7=more than 75% = of leaf area infected.

Disease severity (%) = Sum of (nxv)/Total No. of leaves observed in sample x grading (7) ×100

Where:

n = number of infected leaves in each category.

v = numerical value of each category.

The sum of numerical values were obtained by multiplying the number of leaves (observed in a particular grade) with their respective grading.

Preparation of enzyme extract

Five gram of fresh lettuce leaves treatments in field experiment were taken after 60 days and homogenized in 50ml 0.1M phosphate buffer (pH 7.0). The homogenate was then centrifuged at 10,000 rpm for 20 min, in a refrigerated centrifuge at 0–4°C. The supernatant obtained was referred to as crude extract and stored under in a freeze for enzyme assays of polyglacturonase (PG) and cellulase (Cx). Determination of these enzymes were done as mentioned above.

Statistical Analysis

The data were statistically analyzed according to **Snedecor and Cochran (1980)**.

Correlations between traits were calculated using the Pearson Correlation Coefficient ($P \leq 0.1$). The relationship between salts concentration, activities of cell-wall degrading enzymes, and disease severity on two lettuce cultivars was extrapolated.

RESULTS

Isolation and Identification

Different fungi were isolated from naturally infected lettuce leaves collected from three governorates. Table 2. *Alternaria alternata* (61.11%) showed the highest frequency followed by *Helminthosporium* sp (16.69%), *Stemphylium botryosum* (11.11%) and *Curvularia lunata* (5.56%). Tentative identification was made according to **Neergaard (1945), Hansford (1946)** as well as **Barnett and Hunter, 1972)**.

Table 2. Frequency percentage of fungi isolated from the leaf spot lettuce plants

Isolate fungi	Frequency of the isolated fungi (%)
***Alternaria alternata* (Fr.) Keissler**	61.11
***Helminthosporium* sp**	16.69
Stemphylium botryosum Wallroth,	11.11
***Curvularia lunata.* (Wakker) Boedijn**	5.56
Saprophyte fungi	5.53

Pathogenicity Test of Isolated Fungi on Lettuce Leaves

Four fungal genera associated with lettuce leaves were pathogenic, and causing leaf spot (Fig. 1). The percentage of infection was differed from one pathogen to another. *A. alternata* showed the highest infection (28.8%) followed by *S. botryosum* (19.4%), *Helminthosporium* sp (14.3%), and *Curvularia lunata* (13.8%).

Determination of Enzyme Activity Secreted by the Pathogens *In vitro*

Results in Table 3 show activity of *A. alternata*, *C. lunata, S. botryosum* and *Helminthosporium* sp. enzyme secretion in culture media. The tested fungi differed in their ability to produce pectolytic (PG) and cellulolytic(Cx) enzyme, peroxidase (PO) and polyphenol oxidase (PPO). *A. alternata* and *C. lunata,* gave high PG activity (86.50,79.60). *A. alternate* and *Helminthosporium* sp. gave high level of Cx (80.0, 49.80). The activity of PPO ranged from 0.01-0.03 units/sec/mg and activity of PO ranged from 0.32-0.41 units/ sec/mg.

Effect of salts on fungal growth *in vitro*

The inhibitory effect of salts on *A. alternata, S. botryosum, C. lunata* and *Heliminthosporium* sp. *in vitro* is shown in Table 4 and Fig. 2. Sodium carbonate and ammonium sulfate strongly decreased mycelial growth. Potassium dibasic phosphate and calcium chloride inhibited mycelial growth to a lesser extent. The results presented in Table 4 and Fig. 2 demonstrat that the mycelial growth of *S.botryosum* was inhibited by sodium carbonate, ammonium sulfate, potassium dibasic phosphate and calcium chloride in descending order followed by *A.alternata, Helminthosporium* sp. and *C.lunata,* with the less inhibition of the mycelial growth of *C.lunata* to less inhibition. The 25 mM K_2HPO_4 salt decreased mycelial growth of *S.botryosum* by 72.3% while decreased valued 43.3% in *A. alternata*, 30.0% in *Helminthosporium* sp., and 24.7% in *C.lunata*. The Na_2CO_3 decreased mycelial growth with a percentage 78.5% for *S.botryosum* ,58.9% for *A.alternata*, 58.8% for *Helminthosporium* sp. and 40.0% for *C.lunata.* The fungicide decreased mycelial growth with a percentage ranged between 43.5-87.7% for different fungi.

Microscopic observations on mycelial reaction

The suppression effect of salt on *A.alternata*,*Helminthosporium* sp., *S.botryosum*, *C.lunata* were further investigated using a light microscopic examination of tested fungi. All tested salt at different concentration had varied effect on radial growth and sporulation of the fungi. Mycelia of pathogens treated with highest concentrations of Na_2CO_3 showed morphological abnormal at 25mM such as lysis of hyphae and malformation with decreased spore formation and associated, the mycelium was apparently damaged compared with control ,while control had normal hyphal cell walls and sporulation showed in Fig. 3.

Inhibitory Effect of Salts

Results in Table 5 show different response of tested fungi to salts at significantly level. The EC concentrations EC_{25}, EC_{50}, and EC_{90} values were calculated for each salts against *A. altrnata, S.botryosum, Helminthosporium* sp. and *C.lunata* according to Lpd line program. Inhibitory effect of salts differed significantly between isolates at EC_{50} value. The inhibitory effect of Na_2CO_3 (100%) and $(NH_4)_2SO_4$, (41100%),

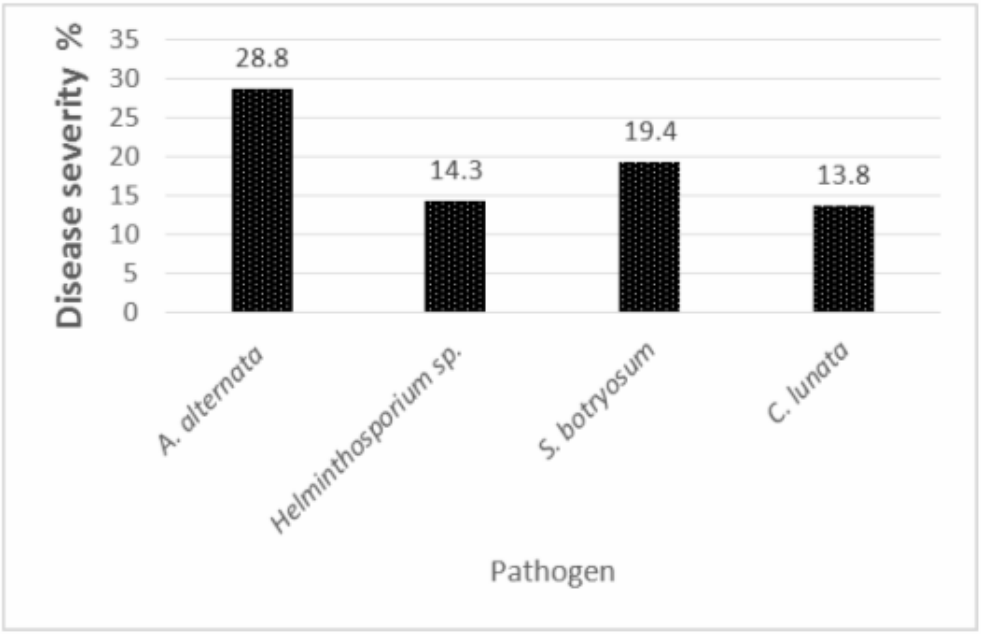


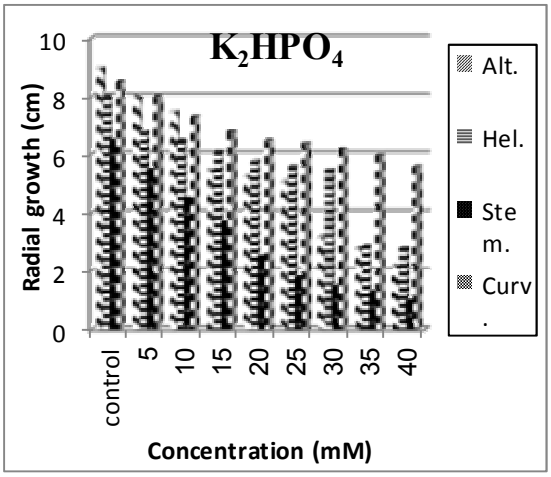
Fig. 1. Pathogenicity test on lettuce leaves as disease severity percentage

Table 3. Activity of enzymes in culture filtrates on Czapek Dox liquid medium after 14 days at 28±2°C

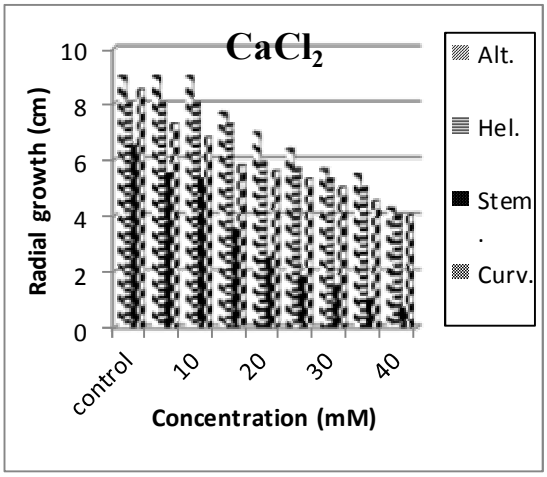
Fungi	Enzyme			
	Loss in viscosity (%) (after 30 min)		Optical density change (after 5 min) units/sec/mg	
	Polyglactoranas	Cellulase	Peroxidase	Polyphenol oxidase
Alternaria alternata	86.50	80.00	0.35	0.03
***Helminthsporium* sp.**	25.50	49.80	0.32	0.03
Stemphylium botryosum	23.00	37.00	0.35	0.01
Curvularia lunata	79.60	39.70	0.41	0.02

Table 4. Effect of different concentrations salts on radial growth of fungi *in vitro*

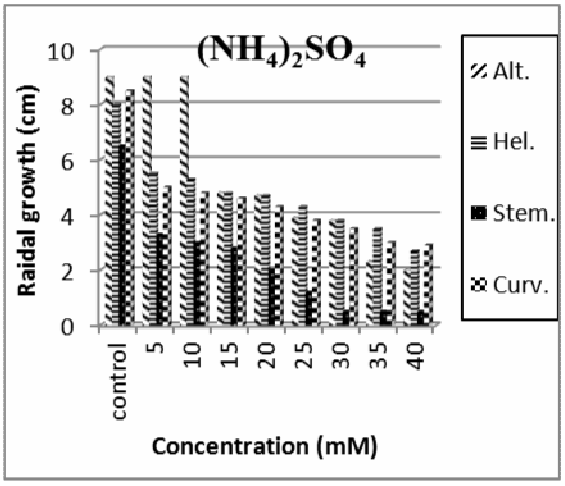
Treatment	Conc.	Radial growth (cm) of the fungi							
		Alternaria alternata		*Helminthsporium* sp.		*Stemphylium botryosum*		*Curvularia lunata*	
		Rate of growth	Inhibition (%)	Rate of growth	Inhibition (%)	Rate of growth	Inhibition (%)	Rate of growth	Inhibition (%)
K_2HPO_4	5 mM	8.0	11.1	6.8	15.0	5.5	15.38	8.0	5.88
	10	7.5	16.7	6.5	18.8	4.5	30.8	7.3	14.1
	15	5.5	38.9	6.1	23.8	3.7	43.1	6.8	20.0
	20	5.3	41.1	5.8	27.8	2.5	61.5	6.5	23.5
	25	5.1	43.3	5.6	30.0	1.8	72.3	6.4	24.7
	30	3.2	64.4	5.5	31.3	1.5	76.9	6.2	27.1
	35	2.8	68.9	2.9	63.8	1.3	80.0	6.0	29.4
	40	2.2	75.6	2.8	65.0	1.0	84.6	5.6	34.1
$CaCl_2$	5 mM	9.0	0.0	8.0	0.0	5.5	15.4	7.3	14.1
	10	9.0	0.0	8.0	0.0	5.3	18.7	6.8	20.0
	15	7.7	18.9	7.3	8.8	3.5	46.2	5.8	31.8
	20	7.0	22.2	5.9	26.3	2.5	61.5	5.6	34.1
	25	6.4	28.9	5.7	28.8	1.8	72.3	5.3	37.6
	30	5.7	36.7	5.3	33.8	1.5	76.9	5.0	41.2
	35	5.5	38.9	5.0	37.8	1.0	84.6	4.5	47.2
	40	4.3	52.2	4.0	50.0	0.7	89.2	4.0	52.9
$(NH_4)_2SO_4$	5 mM	9.0	0.0	5.5	31.3	3.3	49.2	5.0	41.3
	10	9.0	0.0	5.3	33.8	3.0	53.8	4.8	43.5
	15	4.8	46.7	4.8	40.0	2.8	56.9	4.6	45.8
	20	4.7	47.8	4.5	41.3	2.0	69.2	4.3	49.4
	25	3.9	56.7	4.3	46.2	1.2	81.5	3.8	55.3
	30	3.8	57.8	3.8	52.5	0.5	92.3	3.5	58.8
	35	2.3	74.4	3.5	56.2	0.5	92.3	3.0	64.7
	40	2.0	77.7	2.7	66.2	0.5	92.3	2.9	65.9
Na_2CO_3	5 mM	8.5	5.5	7.5	6.3	2.5	61.5	6.3	25.9
	10	8.0	11.1	4.8	40.0	2.1	67.7	6.0	29.4
	15	4.0	55.6	4.5	43.8	2.0	69.2	5.8	31.8
	20	3.8	57.8	4.0	50.0	1.7	73.8	5.5	35.3
	25	3.7	58.9	3.3	58.8	1.4	78.5	5.1	40.0
	30	0.5	94.4	2.4	70.0	0.9	86.2	2.4	71.8
	35	0.5	94.4	0.9	88.8	0.7	89.2	1.4	83.5
	40	0.5	94.4	0.5	93.8	0.5	92.3	1.0	88.2
Fungicide	5 ppm	4.3	52.2	3.1	61.3	0.8	87.7	4.8	43.5
	15	3.5	61.1	2.7	66.3	0.7	89.2	4.2	50.5
	25	3.0	66.7	2.2	72.3	0.6	90.8	3.3	61.1
	30	2.5	72.2	1.8	77.8	0.5	92.3	2.5	70.6
	40	2.0	74.4	1.6	80.0	0.5	92.3	2.0	76.5
	50	0.5	94.4	0.5	93.5	0.5	92.3	1.5	82.4
	100	0.5	94.4	0.5	93.5	0.5	92.3	0.5	91.86
	250	0.5	94.4	0.5	93.5	0.5	92.3	0.5	92.30
Control		9.0	----	8.0	----	6.5	----	8.5	---
LSD at 5%	T= 0.3317 Con .= 0.4195 T x C = 0.9381			T= 0.3099 Con .=0.3921 T x C = 0.8767		T= 0.2137 Con .= 0.2703 T x C=0.6042		T= 0.2602 Con .= 0.3291 T x C= 0.7358	



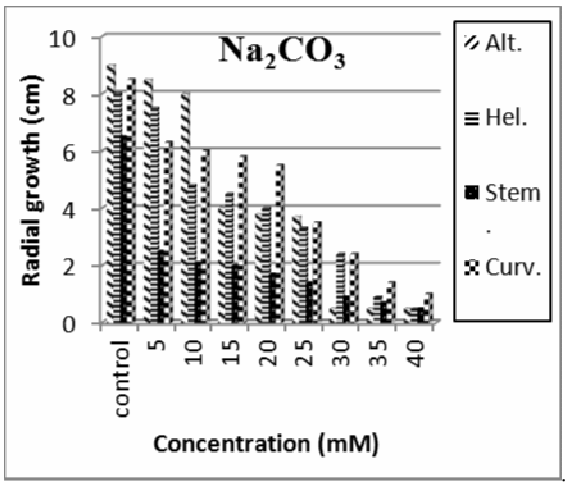
a: Effect of different concentrations of K_2HPO_4 on radial growth of *A. alternata*, *Helminthosporium* sp, *S. botryosum, C. lunata.* .



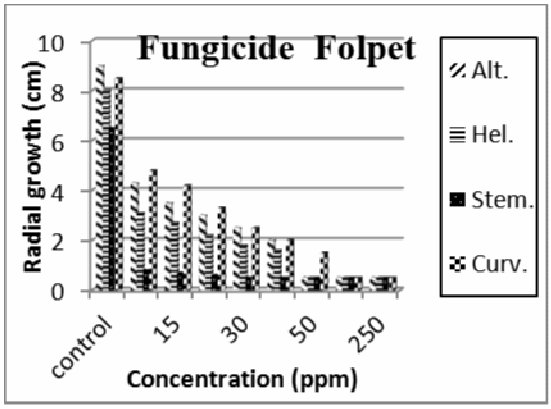
b**:** Effect of different concentrations of $CaCl_2$ on radial growth of *A. alternata*, *Helminthosporium* sp, *S. botryosum, C. lunata..*



c: Effect of different concentrations of $(NH_4)_2SO_4$ on radial growth of *A. alternate, Helmithosporium* sp,*S.botryosum,C.lunata* .



d:Effect of different concentrations of Na_2CO_3 on radial growth of *A. alternate, Helminthosporium* sp,*S. botryosum,C. lunata..*



e: Effect of different concentrations of **Folpet (80%)** on radial growth of *A. alternata*, *Helminthosporium* sp, *S.botryosum, C. lunata.*

Fig. 2. a,b,c,d,e Effect of salts and their concentration on radial growth of *Alternaria alternata*, *Helminthosporium* sp, *Stemphylium botryosum, Curvularia lunata*

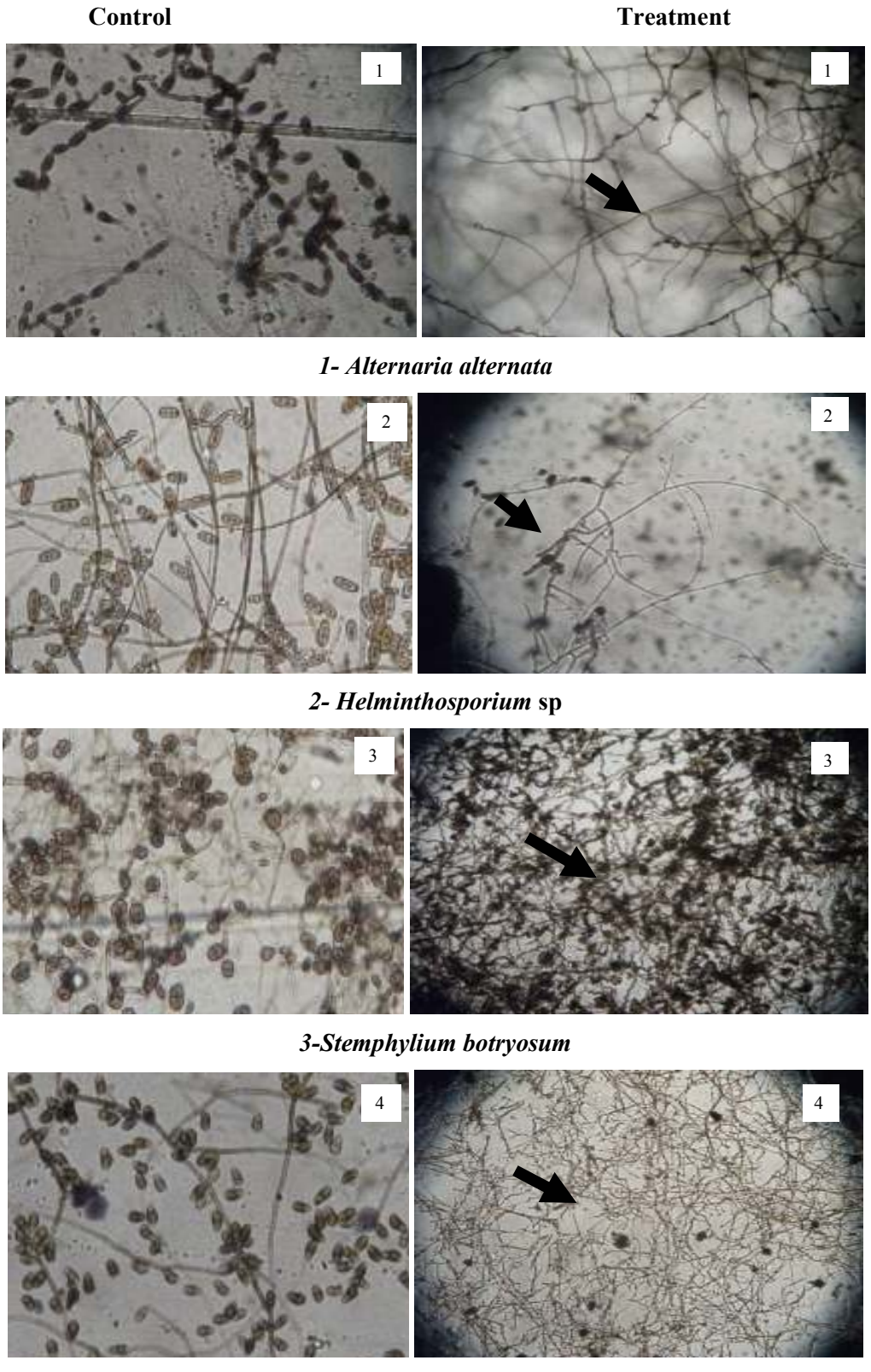


Fig. 3. From left: Pathogens (Control) magnification 200x. From right: arrows showing malformed hyphae and less sporulation after treatment with Na_2Co_3 magnification 200x

Table 5. Inhibitory effect (EC) of salts on mycelial growth of the tested fungi

Salts	EC level (ppm)			Slope (statistical)	Inhibition index*
	EC_{25}	EC_{50}	EC_{90}		
			Alternaria alternata		
K_2HPO_4	1945.462	3933.576	14974.053	2.2076±1925	44.998
$CaCL_2$	2440.099	4592.347	15270.267	24561±0.3960	38.543
$(NH_4)_2 SO_4$	1182.264	2541.574	10880.904	2.0293±0.3651	69.643
Na_2CO_3	1156.707	1770.017	3972.135	3.6508±0.2917	100.0
Folpet 80%	1.6915	5.5762	53.7875	1.3020±0.2068	---
			Curvularia lunata		
Salts		**EC level (ppm)**		**Slope(statistical)**	**Inhibition**
	EC_{25}	EC_{50}	EC_{90}		**index***
K_2HPO_4	4237.082	16620.647	223098.147	1.1363±0.1986	11.428
$CaCL_2$	1299.958	4517.579	48172.707	1.2468±0.1774	42.045
$(NH_4)_2 SO_4$	265.519	1899.396	79848.083	0.7893±0.1407	100.00
Na_2CO_3	880.407	1955.447	9053.285	1.9327±0.1778	96.590
Folpet 80%	1.4792	8.362	224.775	0.8966±0.1137	---
			***Helminthosporium* sp.**		
Salts		**EC level (ppm)**			**Inhibition**
	EC_{25}	EC_{50}	EC_{90}	**Slope(statistical)**	**index***
K_2HPO_4	2339.453	6334.365	42039.133	1.5592±0.1869	27.549
$CaCL_2$	2563.821	4671.624	14607.954	2.5885±0.4031	37.354
$(NH_4)_2 SO_4$	593.103	2786.262	52682.460	1.0039±0.1618	62.630
Na_2CO_3	991.606	1745.045	5107.553	2.7478±0.1982	100.00
Folpet 80%	0.549	3.3259	101.890	0.8623±0.1648	----
			Stemphylium botryosum		
Salts		**EC level (ppm)**		**Slope(statistical)**	**Inhibition**
	EC_{25}	EC_{50}	EC_{90}		**index***
K_2HPO_4	1383.808	2660.181	9209.150	2.33764±1.849	14.803
$CaCL_2$	984.816	1739.242	5124.918	2.7307±0.1961	22.642
$(NH_4)_2 SO_4$	333.955	946.813	6857.898	1.4904±0.2067	41.592
Na_2CO_3	97.760	393.796	5559.311	1.1147±0.1698	100.0
Folpet 80%	0.0	0.0008	14.7982	0.3000±0.2747	----

*Toxicity index= (EC_{50} of the most effective pesticides / EC_{50} of least effective pesticides) x 100

is shown for *A.alternata, Helminthosporium* sp*., S. botryosum* and *C. lunata.* The inhibition effect of $(NH_4)_2 SO_4$, Na_2CO_3 on mycelial growth, varied compared to treatments with $CaCl_2$, K_2HPO_4 on mycelial growth of *S.botryosum, Helminthosporium* sp*., C. lunata*, *A. alternate,* respectively (22.6, 37.4, 38.5 and 44.0)while K_2HPO_4 was effective on mycelial growth of *C.lunata*, *S.botryosum, Helminthosporium* sp. and *A. alternate* (11.4, 14.8, 27.5 and 44.9), respectively compared with fungicide.

Field Experiment

Results in Table 6 showed values of disease severity in field trials of two lettuce cultivars. The most effective management treatment was Folpet 80% fungicide, followed by Na_2CO_3, $(NH_4)_2SO_4$ and $CaCl_2$ at high concentration, respectively (20.9, 26.1, 25.7, 28.6) in Cultivar (Balady) and (23.7, 28.6, 30.5, 30.2) in Cultivar (Dark green), while the lowest effect was recorded for K_2HPO_4 treatment.

Two lettuce cultivars showed different responses to cell wall degrading enzymes. Polyglactornase (PG) and cellulase (Cx) activities were increased with low concentrations. The most effective salt treatment was recorded for $(NH_4)_2SO_4$, CaCl2, Na_2Co_3 and K_2HPO_4 that showed pronounced activity.

The results of the present study demonstrated that the cultivar play a determinant role in the relationship between activity of cell- wall degrading enzymes and disease severity .Thus, the activity of PG was very highly correlated with disease severity (P=0.005) on cultivar balady and only correlated (p= 0.011) on dark green. Cellulase Cx showed very high significant correlation (p=0.000) with disease severity on balady while it was not correlated with disease severity on dark green (Table 7).

DISCUSSION

Foliar diseases of lettuce caused by 16 fungal species are most important and widespread constraint that causes serious yield losses at all growing areas of the world **(Bradley *et al.*, 2009)**.

In this study the predominant fungal pathogens of the crop under the Egyptian condition were identified on lettuce leaves as *A.alternata* (Fr.) Keissler*, Helminthosporium* sp*., S. botryosum* (Wallroth) and *C. lunata* (Wakker).

Recovered fungi were different in their ability to produce PG, Cx, PPO, and PO enzymes. The expressed activity in terms of loss in viscosity produced by PG in tested fungi ranged between (23.0 - 86.5%), compared to Cx activity (37.0 - 80.0%). PPO activity (0.01-0.03 units/sec/mg) and PO activity (0.32-0.41 units/ sec/mg).

The inhibitory effect of salts tested on *A.alternata, S.botryosum, Helminthosporium* sp. and *C.lunata* growth was studied. The salts Na_2CO_3, $(NH_4)_2SO_4$, $(CaCl_2)$ and (K_2HPO_4) varied greatly in recorded EC 50% inhibitory salt concentration (5-40mM), and a complete inhibition was reported for the dilute Flopet 80% fungicide (50 ppm).

The concentration of EC_{25}, EC_{50}, and EC_{90} values were calculated for each salt against *A.alternata, S.botryosum, Helminthosporium* sp. and *C.lunata* according to Lpd line program. Inhibitory effect of salts for growth differed significantly between isolates at EC_{50} value. The inhibitory activity of the tested salts Na_2CO_3, $(NH_4)_2SO_4$, $CaCl_2$, K_2HPO_4 is shown for *A. alternata,* and *Helminthosporium* sp*.,* while the ammonium sulfate the inhibited growth mycelial for *S.botryosum* and *C. lunata* then Na_2CO_3, $CaCl_2$, K_2HPO_4 .(Table 4).

Mycelial growth of pathogenic fungi were inhibiting by treatment with different concentration of tested salts on PDA plate. The sodium carbonate and ammonium sulfate concentration were more effect on malformation hyphae and less sporulation. In this regard the hydric stress has to deal with the increase in osmotic pressure and may therefore change their physiology (**Killham, 1994)**. As well as calcium acetate and calcium chloride did not inhibit growth of *Monilinia fructicola* on PDA as strongly as 4 other calcium salts (calcium oxide, calcium propionate, calcium pyrophosphate, calcium silicate) as reported by **(Biggs *et al*., 1997)**.

In the present work disease(s) control with nonspecific control salts was studied. Plants were sprayed weekly and disease severity was assessed. In general, the most effective control treatment was Na_2CO_3,$(NH_4)_2SO_4$, $CaCl_2$ and K_2HPO_4 that decreased leaf spot severity.

Table 6. Effect of spraying lettuce plants with different salt concentrations on activity of polyglacturonase (PG), cellulase (Cx) and disease severity on two lettuce cultivars

Treatment		Cultivar (Balady)			Cultivar (Dark green)		
		Loss in viscosity (%) (after 30 min)		Disease severity (%) X_3	Loss in viscosity (%) (after 30min)		Disease severity (%) X_6
	Conc. g/l	PG X_1	Cx X_2		PG X_4	Cx X_5	
K_2HPO_4	2	28.1	31.9	32.4	16.9	24.9	34.4
	4	26.8	25.9	29.5	12.2	17.8	29.6
$CaCL_2$	2	32.9	21.9	33.0	20.5	17.7	32.9
	4	26.9	12.8	28.6	15.3	17.8	30.2
$(NH_4)_2 SO_4$	2	33.8	27.1	30.5	31.7	29.4	33.3
	4	21.2	16.4	25.7	28.3	20.4	30.5
Na_2CO_3	2	28.5	28.3	30.5	14.9	18.9	32.4
	4	21.4	17.9	26.1	11.2	16.9	28.6
Folpet 80%	1	12.0	25.9	21.9	4.8	10.9	25.7
	2	7.13	15.8	20.9	2.5	8.4	23.7
Control (infected)	---	38.1	36.0	46.3	39.2	36.3	57.4

Table 7. Correlation between cell-wall degrading enzymes and disease severity on two lettuce cultivars

Enzyme	Cultivar	
	Balady	Dark green
Polyglacturonase	0.780[a]	0.729
	(0.005) [b]	(0.011)
Cellulase	0.915	0.405
	(0.000)	(0.217)

a Linear correlation coefficient ® between PG and DS on cultivar balady. linear correlation was calculated on the results shown in Table 6.

[b] Probability level

Also copper sulphate is a fungicide used to prevent and control plant fungal diseases including powdery mildew, leaf spots, blight and inhibit spore germination and fungal growth **(Meister, 1992)**.

Among the tested salts calcium cations may decrease directly fungal infection by inhibiting fungal growth and inhibiting cell wall degrading enzymes produced by the pathogens. The effects of calcium in decrease spore germination were probably due to toxicity, with high concentrations likely affecting the osmotic balance in fungal cells, commonly Na^+ or Cl^- (**Lauchli and Grattan, 2007).** The response of plant growth to salinity has been characterized as a combination of the following effects: turgor reduction affecting stomata conductance and cell expansion, growth limitation due to the rate of photosynthesis; and/ or accumulation of salts or specific ions affecting the production of particular metabolites (**Munns and Tester, 2008).**

polygalacturonase activity in both plant cultivars under investigation showed apparently similar pattern. They showed significantly high activity. Low Cx activity was recognized in dark green compared to that in balady cultivar. It is well established that most plants have natural inhibitor proteins that slow the hydrolytic activity of PG (**D'Ovidio *et al.,* 2004)**.

Mode of action and regulation of plant PGs was discussed as PGs belong to an enzyme family detected in plants (**Verlent *et al.*, 2005)**, herbivorous insects (**Shen *et al.,* 2003**), and microorganisms such as bacteria, fungi, and nematodes (**Mertens and Bowman, 2011**). Whatever their origin, PGs cleave by hydrolysing the α-(1–4) bonds linking d-Gal-A residues, mainly from the HG linear homopolymer (**Protsenko *et al*., 2008**).

Daniel *et al*. (2002) reported that the polygalacturonase inhibitor proteins (PGIPs) that have been reported to demonstrate both non-competitive and competitive inhibition of PGs **Federici *et al* (2001**). The active site of PG interacts with a pocket containing multiple polar amino acids in *Phaseolus vulgaris* PGIP2 **D'Ovidio *et al.* (2004)** found that phytopathogenic fungi expose plant cell walls to cell wall degrading enzymes like PGs. In response, most plants have natural inhibitor proteins that slow the hydrolytic activity of PG.

The results of the present study indicated that the tested salts gave good control directly after Folpet (80%) as a standard general fungicide against lettuce leaf spots. The salts could be used as effective and safe method for controlling different airborne plant pathogens in addition to, the avoidance of environmental pollution due to planned decrease in the usage of chemical fungicides.

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الأمـــلاح كعوامل لمقـــاومـــة أمـــراض تبقعـــات أوراق الخـــــس

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اجري هذا البحث لدراسة تأثير إستخدام أملاح كبريتات الأمونيوم وكربونات الصوديوم وفوسفات البوتاسيوم وكلوريد الكالسيوم على نمو الفطريات المعزولة من تبقعات أوراق نباتات الخس وهى الترناريا الترناتا وهيلمنثوسبوريوم وكرفيولاريا وستمفيليم، أوضحت النتائج أن كربونات الصوديوم وكبريتات الأمونيوم قللت نمو الفطريات بشده مقارنه فوسفات البوتاسيوم وكلوريد الكالسيوم، أثر إستخدام كربونات الصوديوم بتركيز 25 ملليمول على نمو الفطر الترناريا الترناتا بنسبه 58.9 % والهلمنثوسبوريوم بنسبه 58.8% وستمفيللم بنسبه 78.5% والكرفيولاريا بنسبة 40.0%، كما أدى إستخدام المبيد الفطرى فولبيت بتركيزات مختلفه الى وقف نمو الفطريات المختبره بنسبه تتراوح بين 43.5% و87.5% عند تركيز 5 جزء فى المليون، قدر الأثر المثبط عند 50% EC_{50} على الفطريات فكان كربونات الصوديوم أكثر الأملاح تأثيراً على معدل نمو فطريات الالترناريا والهلمنثوسبوريوم والستمفيليم والكرفيولاريا وأقلهم تأثيراً فوسفات البوتاسيوم، ووجد أن كربونات الصوديوم الأعلى تأثير على مرض تبقعات الأوراق فى الخس حيث تحفز معامله النباتات بالاملاح الى انخفاض شده الاصابه على صنفى الخس تحت الدراسه، وجد تباين بين إفراز إنزيم البولى جلاكتورينيز والسليوليز على صنفي الخس عند معاملتها بالأملاح المختبرة، وأوضحت الدراسه إمكانيه مقاومه تبقعات الأوراق بالأملاح للإقلال من استخدام المبيدات خاصه فى التغذيه بالنباتات الورقية ومن تلك الأملاح كربونات الصوديوم وكبريتات الامونيوم وكلوريد الكالسيوم وفوسفات البوتاسيوم.

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