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Efficacy of repeated spray of neem oil for control of gray mold disease of lentil plants caused by *Botrytis cinerea* and on some of the chemical components of lentil seeds

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ABSTRACT

Neem oil at concentrations of 2.5, 5.0, 7.5 and 10 % were tested *in vitro* using Petri dishes. These concentrations decreased the radial growth of *Botrytis cinerea* by 27.31, 39.54, 94.46 and 100.00%, respectively. Also, Dithane M 45 decreased the fungal growth by 39.00, 49.29 and 100.00% at the concentrations of 50, 150 and 250 ppm, respectively. Field experiments were carried out at Etay El-Baroud Agriculture Research Station Farm, El-Behera Governorate during 2002/03, 2003/04 and 2004/05 growing seasons to find out the effect of neem oil repeated spray for the management of Botrytis gray mold (*B. cinerea*) of lentil (*Lens culinaris*) and on some chemical components of lentil seeds under field natural infection conditions. Treatments were used of water spray (N_0), Neem oil 5 % spray; once only (N_1), twice (N_2), and three times (N_3), 15 days intervals. Five varieties of lentil were sown; Sinai 1, Giza 370, Giza 9, Giza 51 and Giza 4. Sinai 1 was the most sensitive to infection where the disease incidence in the check treatment (N_0) ranged from 66.67 to 56.67 % in seasons 2002/03 and 2003/04, respectively and the disease reduction ranged from 85.00 to 70.58 % in the treatment (N_3). On the other hand, Giza 51 was the most resistance to the infection where the disease incidence in (N_0) treatment ranged from 33.33 to 25.00 % and it decreased to 10.00 and 11.67 % in (N_3) treatment in the above two growing seasons, respectively. Generally, there is a correlation between neem oil repeated spray and the improvement of lentil agronomic characters. Comparative study of repeated spray of neem oil and Dithane M 45 three times cleared that neem oil repeated spray decreased gray mold incidence in Giza 9 and Sinai 1 with 67.74 and 44.44% in season 2003/04 and 55.16 and 25.93% in season 2004/05, respectively. Also, data showed that Sinai 1 had the higher seed yield / feddan, crude protein, phosphorus and potassium seed contents (kg / fed) than the other four varieties, whereas Giza 51 and Giza 4 have the lowest values. Repeated spray of neem oil 5% or Dithane M 45 three times significantly increased the above four parameters over the check treatment.

Keywords: Lentil, Varieties, Neem oil, Gray mold, Alluvial soil, Protein, Potassium, Phosphorus.

INTRODUCTION

Lentil (*Lens culinaris* Medik.) is an important legume crop food especially for cool season (Beniwal *et al.*, 1994). Seed borne *Botrytis cinerea* Pers. Ex. Fr. is associated with number of plant species (Antonov *et al.*, 1997) including chickpea (Burgess *et al.*, 1997) and lentil (Morrall, 1997). High relative humidity and free moisture on plant surfaces are required for conidia of *B.cinerea* to germinate, produce a germ tube, and infect host (Blackman, 1980; Jarvis, 1980; Carre and Coyier, 1984 and Elad, 1989).

Due to the increasing public awareness of the pollutive, residual, carcinogenic and phytotoxic effects of synthetic fungicides (Awuah,1989 and Bankole and Adebanjo,1995), pesticides derived from plant products have been found to be non-phytotoxic, more systemic with little or no mammalian toxicity and easily biodegradable (Fawcett and Spencer, 1970; Rao and Singh, 1992). The neem tree (*Azadirachta indica* A. Juss.) has emerged as the single most effective plant species possessing many pesticidal properties (Koul *et al., 1990).* The bioactive ingredients of neem have been shown to be efficacious in controlling several crop diseases (Lal *et al.*, 1980; Vir and Sharma, 1985; Ahmed and Grainge, 1986; Bhaskaran *et al.*, 1988 and Mathur and Lodha, 1994). Neem has both contact and systemic action in many plants. It coats the leaf surface which in turn prevents the germination of the fungal spores (Anonymous, 2003). Although neem oil has been used for control of wide range of phytopathogenic fungi (Locke, 1995; Bankole, 1997 and Govindachari *et al.*, 1998), concentrations needed for complete field control were shown to be as high as 2% to 10% (Locke, 1995).

This paper has focused on the search for neem oil efficacy against *B. cinerea* which may have potential in gray mold disease control and also, the effect of neem oil repeated spray on crude protein, phosphorus, potassium lentil seed contents.

MATERIALS AND METHODS

Isolation, purification and identification of the pathogen: Lintel plants showing gray mold symptoms were collected from Etay El-Baroud Agric. Res. Station Farm, El-Behera Governorate during 2002 / 03 growing season. The infected capsules were surface sterilized and placed on wetted filter paper in Petri dish at 20 ± 2° C. After 4 days the formed conidia were picked off using single spore method and used for identification according to Morgan (1971) and Munijal (1980).

Laboratory experiment:

Effect of neem oil on fungal growth: Neem oil used in this study was sterilized using Zeits filter and successful emulsifiable concentrations; 2.5, 5.0, 7.5 and 10 % were prepared using Tween 80 (0.05 %) (Marques *et al.*, 2004 and Abo-El Seoud *et al.*, 2005) in PDA medium. At the same time, three concentrations of Dithane M 45® 50, 150 and 250 ppm (Abou-Zeid *et al.*, 1990) were used. Each concentration had five Petri dishes 9 cm. Four mm diameter disks from the growing fungal margin of 8 days old culture were removed and placed in the center of the medium. Growth diameter (cm) of colonies on inoculated plates which incubated at 20±2°C and 12 h photoperiod was measured after 3, 7 and 10 days. Fungitoxicity was expressed in terms of percentage of mycelial growth inhibition after 10 days and calculated as formula (Pandey *et al.*, 1982);

Percentage of mycelial growth inhibition = [(dc – dt) /dc] x 100

Where; dc: Average diameter of fungal colony in the check.
dt: Average diameter of fungal colony in treatments.

Field experiments: Experiments were carried out at Etay El-Baroud Agric. Res. Station during three successive growing seasons; 2002/03, 2003/04 and 2004/05 in a randomized complete block design with three replications. Soil samples were taken from the soil surface layer of the farm (0-30 cm) before planting for physical and chemical analysis, which was done according to Black (1965). The results of soil analysis are shown in Table (1).

Table (1): Physical and chemical analysis of the studied soil.

Seasons	Organic Matter %	$CaCO_3$ %	Particle size distribution				Soil Texture	Available Nutrients ppm		
			Coarse Sand%	Fine Sand%	Silt%	Clay%		N	P	K
2002/03	1.30	3.65	11.00	9.50	38.40	41.10	Clayey	50	6	235
2003/04	1.44	3.40	10.98	9.54	37.58	41.90	Clayey	69	4	250
2004/05	1.34	4.07	11.29	9.78	39.60	39.33	Clayey	77	5	260

Seasons	Ph 1 : 2.5 Soil:water	EC.dS/ m 1 : 5 Soil:water	Soluble cations and anions meq/L							
			Cations				Anions			
			Ca^{++}	Mg^{++}	Na^{+}	K^{+}	CO_3^{--}	HCO_3^{-}	Cl^{-}	SO_4^{--}
2002/03	7.40	0.50	1.50	1.40	1.65	0.20	---	0.40	0.65	3.70
2003/04	7.60	0.30	1.10	1.30	1.60	0.16	---	0.30	0.60	3.26
2004/05	8.46	1.12	0.67	0.34	0.50	0.26	---	0.56	0.29	0.92

Because of lentil plants sensitivity to irrigation, they were sown on ridges. Each experimental plot (5.4 m^2) had three ridges, 60 cm. in between and 3 meters long. Five lentil varieties namely; Sinai 1, Giza 370, Giza 9, Giza 51 and Giza 4 were sown in two sides / ridge with 5 seeds / hill, 10 cm. a part at the 15th of November for the growing seasons.

Evaluation of neem oil repeated spray: This experiment was carried out during 2002/03 and 2003 /04 growing seasons. Each lentil variety was sprayed with neem oil 5% (Locke, 1995 and Bowers and Locke, 2004) emulsified with Tween 80 (0.05%) either once only (N_1), twice (N_2) or three times (N_3). The first spray was carried out at 15th of January and then each 2 weeks. Check treatment (N_0) was sprayed with water and Tween 80 (0.05%). Each treatment had three replicates. At the first of March, 30 lentil plants were taken from each treatment, washed with tap water, surface sterilized with 2% sodium hypochlorite for 2 minutes and finally washed in sterilized water (Barnes and Shaw, 2003). Plants were partially dried between two layers of sterilized cheesecloth, put in 3 moist glass jars and then gray mold incidence was estimated.

At harvest, twenty guarded plants were taken at random on which the following growth characters were recorded: Plant height (cm), straw

weight / 20 plants (g), seeds weight / 20 plants (g), seeds number / 20 plants and 1000 seeds weight (g).

Comparison between the effect of repeated spray of neem oil and Dithane M 45®: This experiment was carried out during 2003/04 and 2004/05 growing seasons. The above five varieties were sprayed either with neem oil 5 % emulsified with Tween 80 or Dithane M 45 (250 g / 100 L water) three times and check treatment received water with Tween 80 and then each 2 weeks. The growth characters mentioned above were recorded in this experiment. Also, samples of lentil seeds were dried, ground and wet digested using H_2SO_4-$HClO_4$ acid mixture and nitrogen, phosphorus and potassium seed contents were determined according to Chapman and Pratt (1961), Watanabe and Olsen (1965) and Richards (1954), respectively.

Data were statistically analyzed according to Snedecor and Cochran (1981). Treatment means were compared by L.S.D at 5% and 1% level of probability.

RESULTS AND DISCUSSION

Laboratory experiments: Figure (1) appears symptoms of gray mold on lentil capsules, leaflets and branches after incubation for 48 h in moist glass jars. It means that plants were naturally infected previously and preparing moist conditions encouraged the growth of *B. cinerea.* This result is in agreement with Beniwal *et al.,* (1994) who reported that gray mold disease development is favored by moist and cool temperature. Also, Blackman (1980) and Aadkavej *et al.,* (2000) cleared that *B.cinerea* is well known to be form latent infection within plants but this mainly is considered be more in the form of a quiescent infection, where the fungus germinates on the plant surface and remains dormant just under the epidermal cells.

Data in Table (2) and Fig (2I and II) show the *in vitro* effect of the tested concentrations of neem oil and Dithane M 45 on the growth of *B. cinerea.* It is clear that neem oil concentrations were active where it decreased the fungal growth after 3, 7 and 10 days compared to check treatment (D). There is a reversible relationship between neem concentration and fungal growth since the growth reduction was 27.31, 39.54, 94.46 and 100.00 % for the concentrations of 2.5, 5.0, 7.5 and 10.0 %, respectively. This result is in harmony with Mello *et al.,* (2005) who reported that neem oil inhibited

the mycelial development and the sclerotia production of *Sclerotinia sclerotiorum*. In contrast, Ali and Hall (1997) reported that neem oil did not show any fungicidal properties.

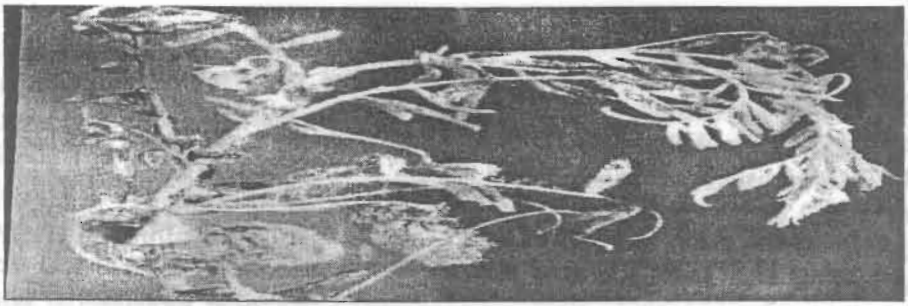

Fig (1): Symptoms of gray mold (*B. cinerea*) on lentil capsules, leaflets and branches after incubation for 48 h in moist glass jars.

On the other hand, Dithane M 45 showed a considerable reduction on the fungal growth where the reduction was 39.01, 49.29 and 100.00 % for the concentration of 50, 150 and 250 ppm, respectively. This effect is in agreement with the finding of Abou Zeid *et al.*, (1990).

Table (2): Effect of different concentrations of neem oil and Dithane M 45 on the growth diameter of *Botrytis cinerea* after 3, 7, and 10 days from inoculation *in vitro*.

Period / Concentration	3 days	7 days	10 days	Mean of Inhibition%
Neem oil				
2.5 %	2.63 (-13.20)*	4.27 (-37.39)	6.18 (-31.33)	27.31
5.0 %	2.03 (-33.33)	3.57 (-47.65)	5.62 (-37.56)	39.54
7.5 %	0.00 (-100.00)	0.00 (-100.00)	1.50 (-83.33)	94.46
10.0 %	0.00 (-100.00)	0.00 (-100.00)	0.00 (-100.00)	100.00
Dithane M45				
50 ppm	1.64 (-45.88)	4.43 (-35.04)	5.75 (-36.11)	39.01
150 ppm	1.60 (-47.20)	3.38 (-50.44)	4.48 (-50.22)	49.29
250 ppm	0.00 (-100.00)	0.00 (-100.00)	0.00 (-100.00)	100.00
Check	3.03	6.82	9.00	–

() * Reduction% $= \frac{\text{Growth diameter in check - Growth diameter in treatment}}{\text{Growth diameter in check}} \times 100$

	$L.S.D_{0.05}$	$L.S.D_{0.01}$
Concentration	0.16	0.21
Period	0.10	0.13
Conc. X Per.	0.27	0.36

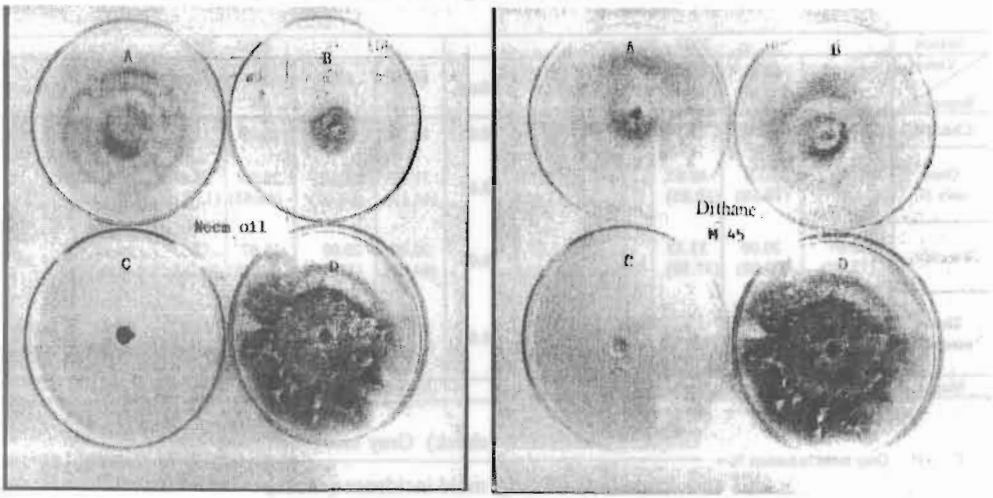

Fig (2): Effect of different concentrations of neem oil and Dithane M 45 on the growth diameter of *Botrytis cinerea*. I - Neem oil; A, B, C, and D refer to 5.0, 7.5, 10.0% and zero, respectively. II- Dithane M 45 A, B, C, and D refer to 50, 150, 250 ppm and zero, respectively.

Field experiments:

Evaluation of neem oil repeated spray: Results in Table (3) show significant differences between varieties, repeated spray and the interaction between them. In both seasons (2002/03 and 2003/04), spray treatments generally decreased the gray mold incidence (GMI). In the first season, data show that in case of variety Sinai 1, treatment (N_0) had the highest gray mold incidence (66.67 %) and Giza 51 had the least one (33.33 %). Generally, it is clear that there is a reversible relationship between the repeated spray of neem oil and gray mold incidence with all varieties used. These results are in agreement with the findings of Ellis and Bardely, (1996) and Anonymous, (2003). They reported that sprayed neem oil on plant leaves provides a protective coating that blocks fungal spores and prevents disease development, and repeated spray every 7–14 days provided protection through the season. In the first season, Sinai 1 had a positive response to neem oil, where gray mold reduction was 34.00, 75.00 and 85.00 % with N_1, N_2 and N_3, respectively.

Table (3): Effect of neem oil 5 % repeated spray on lentil gray mold incidence under field conditions during two successive growing seasons.

Season	2002 - 2003						2003 – 2004					
Variety / Repeating	Sinai 1	Giza 370	Giza 9	Giza 51	Giza 4	Mean	Sinai 1	Giza 370	Giza 9	Giza 51	Giza 4	Mean
Check N_0	66.67	40.00	53.33	33.33	56.67	50.00	56.67	31.67	46.67	25.00	50.00	42.00
Once only N_1	40.00 (34.00*)	33.33 (16.68)	46.67 (18.85)	26.67 (19.98)	46.67 (17.65)	38.67	31.67 (44.12)	31.67 (00.00)	25.00 (46.43)	21.67 (13.32)	35.00 (30.00)	29.00
Twice N_2	16.67 (75.00)	20.00 (50.00)	33.33 (37.50)	13.33 (60.00)	17.65 (17.65)	26.00	20.00 (64.71)	20.00 (36.85)	16.67 (64.28)	13.33 (46.68)	26.67 (46.66)	19.33
Three times N_3	10.00 (85.00)	16.67 (58.33)	13.33 (40.00)	10.00 (70.00)	33.33 (41.19)	16.67	16.67 (70.58)	15.00 (52.64)	13.33 (71.44)	11.67 (53.32)	23.33 (53.34)	16.00
Means	33.34	27.50	36.67	20.83	45.84		31.25	24.59	25.42	17.92	33.75	

( )* : Gray mold reduction % = $\frac{\text{Gray mold incidence (check)} - \text{Gray mold incidence (treatment)}}{\text{Gray mold incidence (check)}} \times 100$

	$L.S.D_{0.05}$	$L.S.D_{0.01}$	$L.S.D_{0.05}$	$L.S.D_{0.01}$
Variety (V)	9.40	12.58	3.72	4.97
Repeating (R)	8.41	11.25	3.32	4.45
V x R	N.S	N.S	7.43	9.94

At the same time, Giza 51 showed highest reduction in case of N_2 and N_3 treatments which were 60.00 and 70.00 %, respectively. Sinai 1 showed a susceptible response to gray mold disease where it was 66.67 and 56.67 % in case of check (N_0) treatment in the two seasons. In contrast, Giza 51 showed some resistance where (GMI) was 33.33 and 25.00 % in the two seasons. Generally, it is clear that repeated spray of neem oil up to three times (N_3) had the best effect for decreasing GMI. This result is in agreement with the finding of Ellis and Bardely (1996) who mentioned that neem oil compounds breakdown fairly quickly in 5 to 7 days in sunlight and in the soil, so it may be need to repeat the application during the growing season. On the other hand, Quarles (2005) cleared that the fungicidal effect of neem oil may be due to its containing sulphur compounds which have their own fungicidal properties.

Tables 4 and 5 appear lentil shoot height (cm) and straw dry weight (g) during the two growing seasons. It is clear that the response of above both parameters varied from variety to another in case of treatment (N_1) where neem oil was sprayed once only, but in case of both treatments (N_2) and (N_3) which neem oil was sprayed twice and three times, the previous two parameters increased with all varieties.

Table (4) : Effect of neem oil 5 % repeated spray on lentil shoot height (cm) and straw dry weight (g) during season 2002– 2003.

Parameter		Shoot height (cm)					Straw dry weight (g)				
Variety / Repeating		Sinai 1	Giza 370	Giza 9	Giza 51	Giza 4	Sinai 1	Giza 370	Giza 9	Giza 51	Giza 4
Check	N_0	29.67*	46.00	44.67	44.67	39.33	20.57	21.10	20.03	13.93	13.37
Once only	N_1	25.33 (-14.63)**	47.00 (+ 2.17)	42.67 (- 4.48)	44.67 (0.00)	46.00 (+16.96)	25.20 (+22.51)	20.10 (- 4.74)	19.37 (- 3.30)	18.83 (+35.18)	17.73 (+32.61)
Twice	N_2	30.33 (+ 2.23)	47.67 (+ 3.63)	44.67 (0.00)	46.33 (+ 3.72)	45.67 (+16.12)	24.10 (+17.16)	21.37 (+ 1.28)	26.20 (+30.80)	17.57 (+26.13)	16.63 (+24.38)
Three times	N_3	36.33 (+22.45)	52.33 (+13.76)	47.67 (+ 6.72)	53.67 (+20.15)	46.67 (+18.66)	30.33 (+47.45)	29.33 (+39.01)	29.43 (+46.93)	20.47 (+46.95)	23.10 (+72.78)
Mean		30.42	48.25	44.92	47.34	44.42	25.05	22.98	23.76	17.70	17.71

* Mean of 20 plants.

( )** = increase % compared to check treatment (N_0).

	$L.S.D_{0.05}$	$L.S.D_{0.01}$	$L.S.D_{0.05}$	$L.S.D_{0.01}$
Variety (V)	2.27	3.04	3 .12	4.17
Repeating (R)	2.03	2.72	2.79	3.73
V x R	N.S	N.S	N.S	N.S

Table (5): Effect of neem oil 5 % repeated spray on lentil shoot height (cm) and straw weight (g) during season 2003– 2004.

Parameter		Shoot height (cm)					Straw dry weight (g)				
Variety / Repeating		Sinai 1	Giza 370	Giza 9	Giza 51	Giza 4	Sinai 1	Giza 370	Giza 9	Giza 51	Giza 4
Check	N_0	26.67*	40.67	38.33	37.67	33.67	16.30	18.07	16.87	10.97	11.33
Once only	N_1	24.00 (-10.01)**	42.00 (+ 3.27)	41.67 (+8.71)	38.67 (+2.66)	37.00 (+9.89)	21.00 (+28.83)	19.20 (+6.25)	17.30 (+2.55)	15.30 (+39.47)	11.97 (+5.65)
Twice	N_2	30.33 (+13.72)	44.00 (+8.19)	42.00 (+9.58)	43.33 (+15.03)	36.67 (+8.91)	21.87 (+34.17)	20.27 (+12.18)	21.53 (+27.62)	14.77 (+34.64)	12.93 (+14.12)
Three times	N_3	34.67 (+29.99)	51.00 (+25.40)	44.67 (+16.54)	48.67 (+29.20)	41.00 (+21.77)	25.67 (+57.49)	25.93 (+43.50)	21.30 (+26.26)	17.33 (+57.98)	18.83 (+66.20)
Mean		28.92	44.42	41.67	42.09	37.09	21.21	20.87	19.25	14.59	13.77

* Mean of 20 plants .
( )**= increase % compared to check treatment (N_0).

	$L.S.D_{0.05}$	$L.S.D_{0.01}$	$L.S.D_{0.05}$	$L.S.D_{0.01}$
Variety (V)	1.70	2.27	0.89	1.18
Repeating (R)	1. 52	2.03	0.79	1.06
V x R	3.40	N.S	1.77	2.37

season ranged from 2.17% (G370, N_1) to 22.45% (S1, N_3) and from 2.66% (G 51, N_1) to 29.99 % (S1, N_3) in the second season. Also, the enhancement of straw weight ranged from 1.28% (G 370, N_2) to 72.78% (G4, N_3) in the first season, and the enhancement of the same parameter ranged from 2.55% (G9, N_1) to 66.20 % (G4, N_3) in the second season.

Table 6 appears lentil seed weight / 20 plants (g), seeds number / 20 plants and 1000 seed weight (g) during the first growing season. The excess of these parameters varied between varieties where it ranged from 4.03% (G370, N_1) to 207.28% (G4, N_3) in case of seed weight / 20 plants, in seed number / 20 plants from 3.54% (G 370, N_2) to 238.43% (G4, N_3) and finally in 1000 seed weight from 0.47% (G4, N_3) to 33.53% (S1, N_3) compared to check treatment (N_0). Data in Table (7) show the same response as it clear in Table (6). The excess in seed weight / 20 plants ranged from 5.20% (G4, N_1) to 155.29 % (G 4, N3), seed number / 20 plants from 3.18% (G4, N_1) to 187.22 % (G 4, N_3) and 1000 seed weight from 2.93% (G9, N_1) to 26.67% (S1,N_3).

These results are in agreement with the findings of Bhattacharya and Goswami (1987) and Akhtar and Mahmood (1995) who reported that neem oil showed a significant improvement on tomato plant growth. Also, Narasimhan *et al.,* (1998) cleared that three formulations of neem oil and pungam oil increased the grain yield of rice.

Generally, it is clear that G9, G51 and G4 which had the least growth parameters appeared the highest response to neem repeating spray, where they have the best increment in the seed weight / 20 plants and seed number / 20 plants compared to check treatment (N_0). These results may be due to the improving effect of neem oil on lentil growth. This conclusion is in agreement with Ellis and Bardley (1996) who reported that neem oil acts as growth regulator.

Comparison between the effect of repeated spray of neem oil and Dithane M 45: Fig. 3 and 4 appear the effect of repeated spray of neem oil and Dithane M 45 three times in the two growing seasons on GMI and some agronomic characters of the five lentil varieties mentioned before under field conditions. Generally, from Table 8 and Fig. 3A and 4A it is clear that repeated spray of neem oil or Dithane M 45 decreased GMI compared to check treatment (N_0), but Dithane had the best effect.

Table (6): Effect of neem oil 5 % repeated spray on seed weight / 20 plants (g), seed number / 20 plants and 1000 seed weight (g) during season 2002– 2003.

Parameter	Seed weight / 20 plants					Seed number / 20 plants					1000 seed weight (g)				
V / R	Sinai 1	Giza 370	Giza 9	Giza 51	Giza 4	Sinai 1	Giza 370	Giza 9	Giza 51	Giza 4	Sinai 1	Giza 370	Giza 9	Giza 51	Giza 4
Check N_0	33.37	16.63	9.73	4.50	5.63	752.3	678.0	335.3	147.3	194.3	44.26	21.64	21.70	24.19	23.22
Once only N_1	42.70	15.43	17.30	9.47	5.37	810.7	573.3	754.0	351.7	203.0	52.55	22.62	22.28	24.16	21.59
	+27.9*	-7.2	+77.8	+110.	-4.6	+7.8	-15.4	+124.	+138.	+4.5	+18.7	+4.5	+2.7	-0.1	-7.0
Twice N_2	50.20	17.30	21.60	14.43	15.07	918.3	702.0	988.3	592.3	563.0	54.66	22.27	25.08	24.59	23.40
	+65.4	+4.0	+122.	+221.	+168.	+22.1	+3.5	+195.	+302.	+190.	+23.5	+2.9	+15.6	+1.7	+0.8
Three times N_3	56.03	25.67	26.63	13.37	17.30	948.0	963.7	1083.6	494.7	657.7	59.10	24.38	24.35	24.56	23.33
	+67.9	+54.4	+174.	+197.	+207.	+26.0	+42.1	+223.	+236.	+238. +238.ii	+33.	+12.7	+12.2	+1.5	+0.5
Mean	45.65	18.76	18.82	10.44	10.87	857.3	729.3	790.3	396.5	404.5	52.64	22.73	23.75	24.38	22.89

* increase % compared to check treatment (N_0).

Variety (V) Repeating (R)

	L.S.D $_{0.05}$	L.S.D $_{0.01}$	L.S.D $_{0.05}$	L.S.D $_{0.01}$	L.S.D $_{0.05}$	L.S.D $_{0.01}$
Variety (V)	1.71	2.29	24.80	33.19	3.08	4.12
Repeating (R)	1.53	2.05	22.19	29.68	N.S	N.S
V x R	3.43	4.59	49.61	66.37	N.S	N.S

Table (7): Effect of neem oil 5% repeated spray on lentil seed weight / 20 plants, seeds number / 20 plants and 1000 seed weight (g) during season 2003– 2004.

Parameter	Seed weight / 20 plants					Seed number / 20 plants					1000 seed weight (g)				
V / R	Sinai 1	Giza 370	Giza 9	Giza 51	Giza 4	Sinai 1	Giza 370	Giza 9	Giza 51	Giza 4	Sinai 1	Giza 370	Giza 9	Giza 51	Giza 4
Check N_0	29.00	14.80	9.97	6.77	5.77	708.7	511.0	472.7	204.7	209.7	42.08	20.29	22.21	24.37	21.83
Once only N_1	30.87	15.80	13.67	10.17	6.07	752.7	490.7	638.7	380.0	216.3	46.10	21.93	22.86	25.26	21.55
	+6.5*	+6.8	+37.1	+50.2	+5.2	+6.2	-3.9	+35.1	+85.7	+3.2	+9.6	+8.1	+2.9	+3.7	-1.3
Twice N_2	42.90	18.80	18.87	13.03	9.80	827.3	579.7	809.7	502.0	407.7	48.39	23.04	24.23	25.60	22.83
Three times N_3	+47.9	+27.0	+89.3	+92.5	+69.8	+16.7	+13.4	+71.3	+145.3	+94.4	+15.0	+13.6	+9.1	+5.1	+4.6
	50.40	23.20	23.17	14.67	14.73	875.3	792.0	910.7	514.3	602.3	53.30	24.80	24.86	26.19	24.25
	+73.8	+56.8	+132.4	+116.7	+155.3	+23.5	+55.0	+92.7	+151.3	+187	+26.7	+22.2	+11.9	+7.5	+11.1
Mean	38.29	18.15	16.42	11.16	9.09	791.0	593.4	707.9	400.3	359.0	47.47	22.52	23.54	25.36	22.62

* increase % compared to check treatment (N_0).

Variety (V) Replication (R)

	L.S.D $_{0.05}$	L.S.D $_{0.01}$	L.S.D $_{0.05}$	L.S.D $_{0.01}$	L.S.D $_{0.05}$	L.S.D $_{0.01}$
Variety (V)	1.92	2.57	20.92	27.99	1.01	1.14
Repeating (R)	1.72	2.30	18.71	25.03	0.90	1.21
V x R	3.85	5.15	41.83	55.97	2.02	2.70

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Table (8): Response of GMI and some agronomic characters of the five tested lentil varieties to repeated spray of neem oil (N) and Dithane M 45 (D) compared to check treatment (N_0) during 2003/2004 and 2004/2005 growing seasons.

Variety	Sinai 1		Giza 370		Giza 9		Giza 51		Giza 4		Mean	
Season / Parameter	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd
GMI												
N	-4.44*	-25.93	-28.57	-17.38	-67.74	-55.16	-29.40	-23.07	-34.37	-18.19	-40.90	-27.95
D	-70.38	-48.16	-38.09	-56.51	-70.97	-65.51	-64.70	-53.85	-49.99	-54.54	-58.83	-55.71
P L												
N	- 7.50	+ 4.62	+10.15	+ 6.97	+ 0.81	+14.15	0.00	+ 5.51	+37.27	+23.87	+ 8.15	+11.02
D	+18.75	+27.69	+20.30	+28.70	+ 7.38	+17.95	+ 6.16	+23.87	+22.56	+23.97	+15.03	+24.44
St W												
N	+32.38	+39.75	+22.48	+11.05	+28.91	+25.12	+66.18	+42.84	+58.39	+ 7.31	+41.67	+25.21
D	+ 7.16	+30.32	+22.48	+18.17	+30.12	+20.03	+71.14	+60.61	+66.53	+20.65	+39.49	+29.96
S N												
N	+11.59	+ 4.69	+ 5.15	- 2.41	+141.37	+60.91	+67.24	+66.39	- 2.40	- 3.57	+44.59	+25.20
D	+26.83	+8.64	+75.78	+62.74	+121.92	- 3.37	+55.05	- 9.34	+16.35	+12.56	+59.19	+14.25
S W												
N	+35.73	+17.96	+11.78	+26.76	+49.95	+52.73	+70.38	+75.04	+53.12	+32.74	+44.19	+41.05
D	+49.96	+38.20	+55.22	+52.68	+44.16	+65.25	+62.26	+119.37	+85.45	+95.27	+58.61	+74.15
S I												
N	+26.51	+21.26	+14.29	+ 5.77	+12.09	+ 0.37	+ 4.77	+ 0.13	+10.28	- 1.92	+13.59	+ 5.12
D	+29.85	+23.92	+21.41	+16.10	+31.32	+12.87	+ 9.94	+ 9.13	+45.44	+44.86	+27.59	+21.38

*Reduction% = [(vc – vt) /vc] x 100

vc = value of check treatment , vt = value of sprayed treatments , GMI : Gray Mold Incidence , P L : Plant Height (cm) , St W : Straw Weight (gm) , S N : Seeds Number / 20 plants , S W : Seeds Weight (gm) / 20 plants , SI : Seed Index (1000 seeds weight g) , 1st : 2003/2004 growing season , 2nd : 2004/2005 growing season

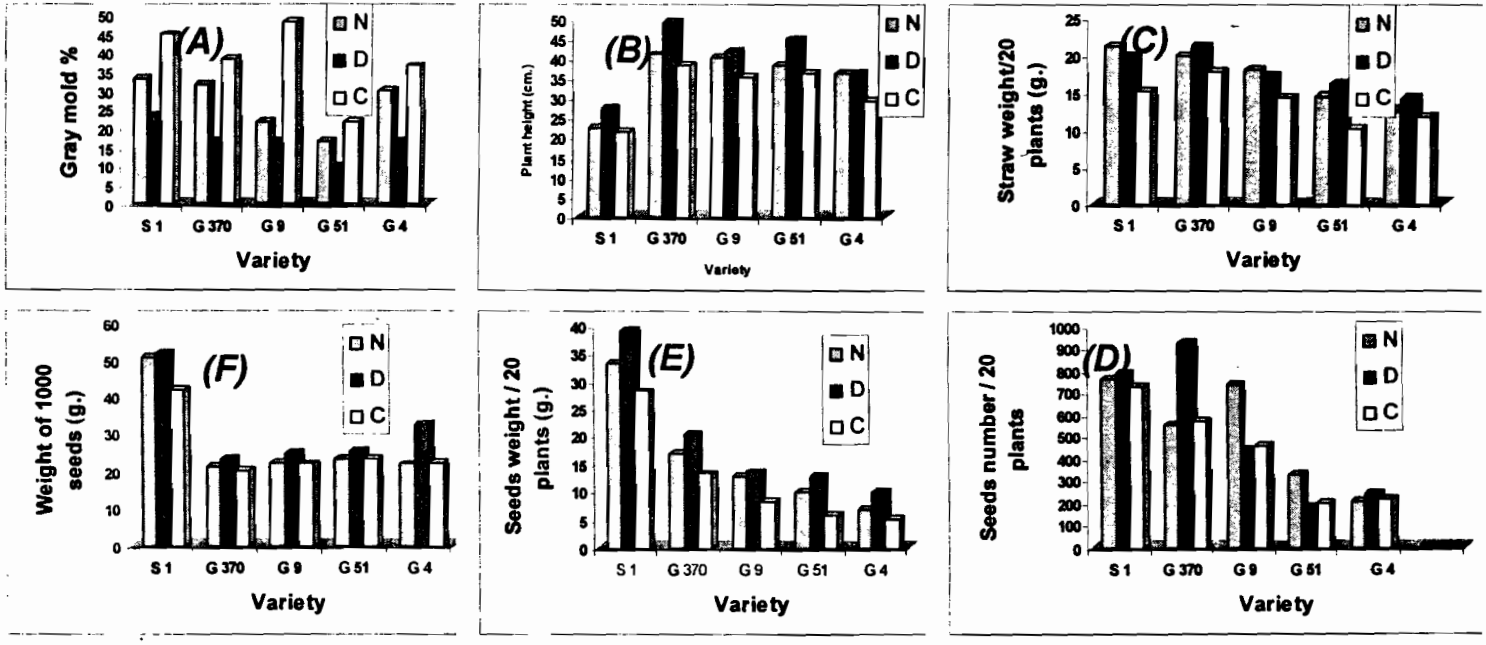

Fig. 3 : Effect of Neem oil (N) and Diathane M 45 (D) as foliar spray,three times,on lentil gray mold%(A),plant height cm. (B),straw weight/20 plants g.(C),seeds number/20 plants(D),seeds weight/20 plants g.(E) and weight of 1000 seeds g.(F) in 2004/2005.

In case of Dithane treatment the most reduction cleared with G9 and S1 (70.97 and 70.38 %) in the first season and G9 and G370 (65.51 and 56.51 %) in the second season, and the average effect was 58.83 and 5.71 % in the two seasons, respectively. On the other hand, the best effect of neem oil was cleared with G9 and S1 in the two growing seasons (67.74 and 44.44 %) and (55.16 and 25.93 %) and the average was 40.90 and 27.95 %, respectively. In this respect, many investigators as Lakshmanan *et al.*, (1990) and Mirza *et al.*, (2000) tested the effect of neem oil on some pathogenic fungi to control *Thanatephorus cucumaris* and *Phytophthora infestans*, respectively. Repeated spray of neem oil or Dithane M 45 improved the agronomic characters of the above tested five lentil varieties as it clear in Fig. 3 and 4 B, C, D, E and F. Generally, the response of some growth parameters compared to check treatment (N_0) is summarized in Table (8).

Seed contents of protein, phosphorus and potassium: Data presented in Table (9) clearly indicated that the different varieties have significant differences on lentil seed yield / fed (kg), crude protein, phosphorus and potassium contents in both seasons. Sinai 1 recorded the highest values for the above four parameters, whereas Giza 51 and Giza 4 recorded the lowest values and the differences between varieties were significant in the two seasons. Generally, Sinai 1 had the first grade followed by Giza 370, Giza 9, Giza 51 and finally Giza 4. The highest seed yield of Sinai 1 might be due to its large seed size and at the same time, its large vegetative growth reflected on its suitable synthesis and building metabolites and these two reasons caused the seed high levels from protein, phosphorus and potassium contents. Such variations in characters between varieties might be reflected by the deficiency of the plant in building metabolites or might be ascribed to the gentical differences. These results are in agreement with the findings of Mokhtar (1993) and Magawer (1990 and 1998) on chickpea.

Also, data clear that the repeated foliar spray three times with Dithane or neem oil increased the four parameters mentioned before significantly over the check treatment. The excess % in the Dithane treatment for all varieties was 51.48, 49.2, 52.8 and 49.0 % in the first season and 54.5, 54.3, 43.4 and 58.0 % in the second season, respectively. These results are in harmony with the results of Shalaby (1997) who reported that sesame seeds treated with Vitavax before sowing in soil infested with *Rhizoctonia solani* stimulated weight of 1000 seeds, number of capsules/ plant, total protein and oil content of seeds over the other treatments. In case of neem treatment

Table (9): Effect of foliar application of Dithane M 45 and neem oil three times on seed yield / fed. (Kg), protein, phosphorus and potassium contents in some lintel varieties seeds during 2003 / 2004 and 2004 / 2005 seasons.

Variety	Treatments	Seed yield/fed (kg)		Seed contents Kg / fed.					
				Crude protein		Phosphorus		Potassium	
		1st	2nd	1st	2nd	1st	2nd	1st	2nd
Sinai 1	Control	708.3	834.4	219	230	2.62	2.69	9.07	10.30
	Dithane	1030.4	980.0	296	294	3.99	3.74	13.17	14.23
	Neem	958.0	736.4	314	280	3.78	3.25	12.73	12.53
	Mean	*898.9*	*850.3*	*276*	*268*	*3.46*	*3.23*	*11.66*	*12.36*
Giza 370	Control	378.0	422.8	84	75	1.10	0.97	4.51	4.17
	Dithane	588.0	509.6	178	157	1.76	1.59	6.60	6.53
	Neem	423.0	333.2	144	146	1.34	1.36	5.43	5.57
	Mean	*462.9*	*421.9*	*135*	*126*	*1.40*	*1.31*	*5.51*	*5.46*
Giza 9	Control	274.4	319.2	83	64	0.71	0.56	3.33	2.63
	Dithane	394.7	341.6	107	95	1.18	1.05	4.60	4.43
	Neem	411.7	210.0	135	108	1.46	1.16	5.57	4.53
	Mean	*360.3*	*290.3*	*108*	*89*	*1.12*	*0.92*	*4.50*	*3.87*
Giza 51	Control	134.4	254.8	41	44	0.39	0.43	1.50	1.73
	Dithane	216.0	316.4	51	77	0.56	0.84	2.43	3.87
	Neem	227.0	145.6	57	64	0.69	0.80	2.60	3.20
	Mean	*192.3*	*238.9*	*50*	*62*	*0.55*	*0.69*	*2.18*	*2.93*
Giza 4	Control	109.2	170.8	37	44	0.26	0.31	1.20	1.50
	Dithane	202.0	248.9	61	83	0.52	0.91	2.40	3.07
	Neem	158.0	126.0	50	55	0.48	0.54	1.80	2.23
	Mean	*156.4*	*181.9*	*49*	*60*	*0.42*	*0.59*	*1.80*	*2.27*
L.S.D 0.05 Varieties		38.77	39.20	13.48	16.84	0.13	0.13	0.49	0.52
Treatments	Control	320.9	310.2	92.9	91.5	1.02	0.99	3.92	4.07
	Dithane	486.1	479.3	138.6	141.2	1.55	1.42	5.84	6.43
	Neem	435.4	400.4	140.0	130.5	1.60	1.63	5.63	5.63
L.S.D 0.05 Treatments		30.03	30.36	8.73	8.17	0.10	0.10	0.38	0.40
L.S.D 0.05 Var. X Treat.		67.14	67.89	19.52	18.27	0.23	0.22	0.84	0.90

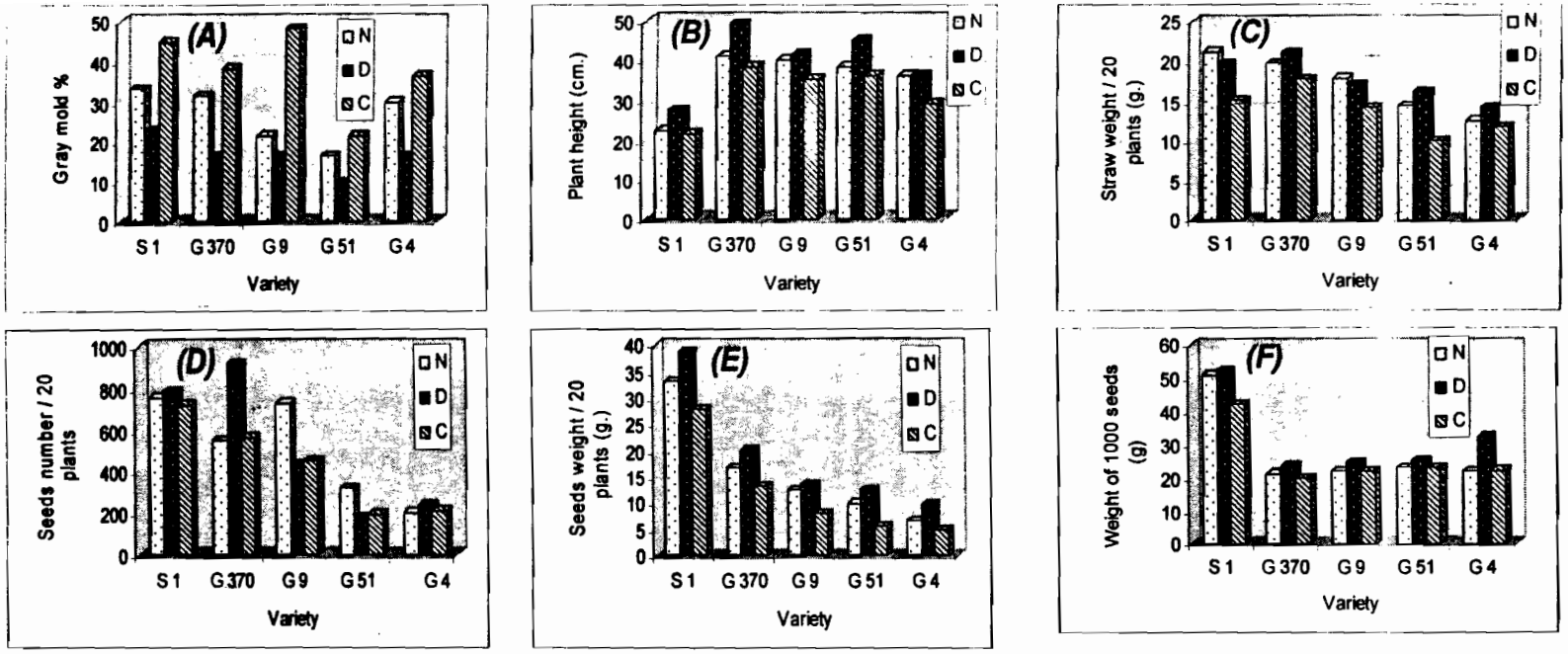

Fig. 4: Effect of Neem oil (N) and Dithane M 45 (D) as foliar spray,three times,on lintel gray mold %(A), plant height cm.(B),straw weight/20 plants g.(C),seeds number/20 plants(D), seeds weight/20 plantsg.(E) and weight of 1000 seeds g.(F)in 2004/2005.

the same table appears that, the above parameters increased with averages 35.7, 50.7, 56.9 and 43.6 in the first season and 29.1, 42.6, 64.6 and 38.3 % in the second season over the check treatment. Finally, the interaction between varieties and treatments (repeated spray with Dithane or neem oil) was significant. It may be concluded that, repeated spray with Dithane M 45 or neem oil 5% three times had a positive effect on lentil seed yield and its chemical components, *i.e,* crude protein, phosphorus and potassium contents. This positive effect may be due to the effect of these chemicals on lentil growth parameters and/or on modifications in the physiological processes which may be induced in lentil plants.

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فاعلية تكرار رش زيت النيم علي مكافحة العفن الرمادي في العدس الذي يسببه فطر بوترايتس سينيريا وعلي بعض المكونات الكيميائية لبذور العدس

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[2] معهد بحوث الأراضي والمياه والبيئة – مركز البحوث الزراعيه – الجيزه

أجري هذا البحث في محطة البحوث الزراعيه بإيتاي البارود فتم إختبار زيت النيم بأربعة تركيزات 2,5، 5,0، 7,5، 10,0% معمليا بإستخدام أطباق بتري وقد وجد أن هذه التركيزات لها تأثير فعال في إنقاص النمو القطري لفطر بوترايتس سينيريا بنسبة 27,31 ، 39,54، 94,46، 100,00% علي الترتيب. أيضا وجد أن مبيد دياثين م 45 أنقص النمو القطري للفطر بنسبة 39,00، 49,29، 100,00 % في حالة التركيزات 50، 150، 250 جزء في المليون علي التوالي.

كما أجريت تجربه حقليه لدراسة تأثير تكرار رش زيت النيم 5% علي نسبة الإصابه بالعفن الرمادي في العدس وكذلك علي بعض الصفات المحصوليه للعدس وذلك تحت ظروف العدوي الطبيعيه بالحقل. كانت معاملات الرش المستخدمه هي : الرش بالماء، رش زيت النيم مره واحده، رش زيت النيم مرتين، رش زيت النيم ثلاث مرات بين كل رشه وأخري 15 يوم وذلك علي خمسة أصناف من العدس هي سينا 1 ، جيزه 370 ، جيزه 9 ، جيزه 51 ، جيزه 4 . وقد وجد أن الصنف سينا 1 أكثر حساسيه للاصابه بالعفن الرمادي حيث كانت نسبة الإصابه في المعامله المرشوشه بالماء تتراوح بين 66,67 – 56,67% في موسمي الزراعه 2002/2003 ، 2003/2004 وقد أنخفضت الإصابه بنسبة 85,00 – 70,58% في معاملة الرش بالنيم ثلاث مرات مقارنة بمعاملة الرش بالماء (المقارنه)0 من ناحيه أخري فقد كان الصنف جيزه 51 أكثر مقاومة للاصابه حيث كانت نسبة الإصابه في معاملة الرش بالماء تتراوح بين 33,33 – 25,00% في موسمي الزراعه وقد إنخفضت إلي 10,00-11,67% في معاملة الرش بزيت النيم ثلاث مرات. وبصوره عامه فهناك ترابط بين تكرار الرش بزيت النيم وتحسن الصفات المحصوليه للعدس.

كذلك فقد أجريت تجربه حقليه أخري لمقارنة تأثير رش زيت النيم 5% أو دياثين م 45 (250 جم/100لتر) ثلاث مرات ووجد أن رش زيت النيم أنقص نسبة الإصابه بالعفن الرمادي في صنفي جيزه 9 ، سينا 1 بنسبة 67,74–44,44 % في موسم 2003/2004 ، 55,16- 25, 93% في موسم 2004/2005 . كما بينت النتائج أن الصنف سينا 1 الأكثر في محصول البذور/فدان، محتوي البذور من البروتين الكلي، الفوسفور، البوتاسيوم عن الأربعة أصناف الأخري. كما أن تكرار الرش بزيت النيم أو دياثين م 45 ثلاث مرات أدي إلي زيادة قيم المقاييس الأربعه السابقه عن معاملة المقارنه لجميع الأصناف.