DORMANCY BREAKING OF SOUR ALMOND SEEDS AND EVALUATION OF BIOTIC AND ABIOTIC ELICITORS FOR INDUCING SYSTEMIC RESISTANCE AGAINST ROOT-KNOT NEMATODES (*MELOIDOGYNE SP.*) IN RELEASED SEEDLINGS

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Abstract

In a Green –House experiments the effect of gibberellic acid at different concentrations on sour almond (*Prunus amygdalus* Batsch.) rootstock concerning percentage of radicle protrusion; stratification period; plant height; number of leaves and plant diameter were determined during 2014 and 2015 seasons. Also, the plant growth promoting rhizobacteria *Serratia marcescens* and the alga *Spirulina platensis* {as biotic elicitors} compared with, Trafose K (1) and white willow *Salix alba* extracts {as abiotic elicitors} soil treatments were tested for induction of systemic resistance (SR) in sour almond plants against root knot nematodes. In relation with nematodes population as juveniles in the soil; galls in roots; females; egg masses in roots were counted. Growth parameters plant, such as heights, fresh weight of roots and shoot were recorded .Also, some chemicals components in the plant, such as phenolic; proline ; Salicylic acid and catalase and peroxidase activity were determined. Results indicated that GA_3 at 1000 p.p.m. achieved the best effect on percentage of radicle protrusion. Data revealed that using *S. marcescens* recorded 40%, 58.3% and 75% reduction in numbers of *Meloidogyne sp.* juveniles in the soil, number of galls and number of females in root respectively in season 2014. While application of *S. platensis* caused 55.6 %, 62.5% and 33.3% reduction of *Meloidogyne sp.* respectively in season 2015. Using *S. marcescens*, *S. platensis*, Trafose K (1) and Salix extract resulted in 60%, 40%, 80% and 20% reduction in the number of development stages, respectively in season 2014, 75%, 50%, 25% and 25% reduction in numbers of development stages, respectively in season 2015. *S. marcescens* inoculants and Salix extract improved plant health by increasing vegetative growth parameters similar to control values. The beneficial effects of these treatments extended to increasing not only total phenol and free proline but also the activities of catalase and peroxidase enzymes in comparison with control plants. All tested elicitors showed significant increasing Salicylic acid (SA) levels when being compared with control. It can be recommended that using GA_3 at 1000 ppm before stratification is necessary to breaking seed dormancy and enhancing radicle protrusion of sour almond rootstock. Also, using *(S. marcescens*, *S. platensis*, as biotic inducers or white willow extracts as natural inducers) as soil treatments increased the induction of systemic acquired resistance

(SAR) in the roots of infected with *Meloidogyne sp*. sour almond rootstock.

Key words: Sour almond ' Gibberellic acid, induced resistance, *Meloidogyne sp.*, Phenols, proline; Salicylic acid. Catalase, peroxidase activity.

INTRODUCTION

Almond (*Prunus amygdalus* Batsch.) is an extensively grown nut crop at regions with a Mediterranean climate (Zacheo *et al.,* 2000). In Prunus species, seed dormancy is an adaptation mechanism to delay germination after the seed has been shed from the tree till the appropriate time for germination. The plant hormones gibberellins (GA_s) and abscisic acid (ABA) are involved in seed dormancy and germination. ABA is involved primarily in regulating seed germination and is a potent germination inhibitor. Additionally, an increase in GA concentration during cold stratification or an increase in GA sensitivity may induce seed germination in many dormant species (HU *et al.,* 2012). Abou-Aly *et al* (2015) reported that root-knot nematodes are serious pathogens that cause severe damage to many plants. A number of constraints are responsible for lowering the production and quality of fruits. Management of nematode associated with almond is of great importance especially as the export of dry fruits is increasing in international markets (Azim, 2015). Cyanobacteria produce compounds, such as Acetamide, hexamethyl, methoxy phenyl, phenol, and others, which are normally toxic to the root-knot nematode. Likewise, cyanobacteria such as *Spirulina, Microcystis*, *Anabaena*, *Nostoc* and *Oscillatoria* produce a great variety of secondary metabolites (Gerwick *et al.,* 2001). Phosphites application to plants for induction of resistance is related to changes in pectin levels and activity of enzymes related to the cell wall structure (Olivieri *et al* 2012). Salicylic acid (SA) was discovered in the extracts of willow (Salix) tree bark and has been used as anti-inflammatory drug since the 18th century. It is a hydroxyl group bearing phenolic compound. Phenolic compounds including SA play important roles in lignin biosynthesis, act as allelopathic compounds, and regulate plant responses to abiotic stimuli and pathogen attacks (Vlot *et al*., 2009). The establishment of Systemic acquired resistance (SAR) is associated with the accumulation of pathogenesis-related (PR) proteins, salicylic acid (SA) and jasmonic acid (JA) throughout the plant.

Systemic resistance against root knot nematode infection has been documented using biological, chemical and natural inducing agents, therefore the present study aimed to achieve the following objectives: - Improving germination and investigating the effect of gibberellic acid pretreatment and stratification on sour almond seed

dormancy termination, and evaluating the potential of using *Serratia marcescens*, *Spirulina platensis*, Trafose K(1) and Salix water extract for the induction of systemic resistance and increasing tolerance of sour almond seedlings against infection with root-knot nematodes.

MATERIALS AND METHODS

A- Effect of gibberellic acid pre-treatment combined with stratification on sour almond seeds:

This study was carried out at Horticulture Research Institute, Agriculture Research Center (A.R.C.), during two successive seasons (2014and 2015). Forty mature seeds of sour almond *Prunus amygdalus* were used and divided in four replicates for each treatment. Seed coats were removed before immersion in the following solutions for 24 hours before stratification.

1- H_2O (control).
2- GA_3 at 500 ppm.
3- GA_3 at 1000 ppm.

Both the treated seeds and the control ones were mixed with the fungicide Vitavax at the rate of 3 g/1 kg seeds, then kept in polyethylene bags full with as peat-moss and stratified on 1st December in a refrigerator at 5^0C (Hartman and Kester, 1978). After taking stratification period in each treatment which mentioned in these results seeds were planting in plastic bags full of (sand: peat-moss 1:1) and adopted in complete randomly design in the greenhouse.

Data were recorded for the following parameters of the three months in each treatment after seed sowing. Plant height, leaves number and plant diameter, were measured.

B- Root-knot nematodes preparation:

The nematode population used in this study was from a greenhouse culture maintained at the Plant Pathology Research, *Meloidogyne sp* was reared in a green house on tomato plants. Eggs of *Meloidogyne sp.* were extracted from roots in 0.5 sodium hypochlorite (Hussey and Barker, 1973) and caught on a 25 m sieve. Second stage juveniles (J_2s) were hatched from these eggs on Baermann funnels and only (J_2s) after 2 days from hatching was used for experimentation.

C– The effect of biotic and abiotic compounds for induction of systemic resistance and increasing tolerance against infection with root-knot nematodes.

This study was carried out during season (2014- 2015). Four month old sour almond seedling uniform in size and vigor as much as possible planted in plastic bags were infected with root-knot nematodes.

*** Green house experiment:**

Twenty five seedlings were inoculated with 3000 second stage juveniles of *Meloidogyne sp*, and five plastic bags with no inoculum of *Meloidogyne sp* were used as the check treatment. Seven days later, plastic bags were divided to six groups and adopted in complete randomly design. Each group contained five seedlings were assigned as follows:-

1. *Serratia marcescens* 2 ml /plant. **2**- *Spirulina platensis* 4 ml /plant.

3- Trafose K (1) 4ml /plant. **4** - Salix water extract 2 ml/plant.

5 - Nematodes only. **6** – Control.

These treatments were applied in the soil in plastic bags.

*** Source and application methods of inducers:**

Serratia marcescens and *Spirulina platensis* were obtained From Biofertelizer production unit, Soil, Water and Environment Research Institute, Agriculture Research Center, Giza Egypt. The bacterium *S. marcescens* was cultured on suitable media such as Nutrient Agar (NA) then transferred to Nutrient Broth-medium and shaken for 48 h. The resulting suspension was centrifuged for 20 min at 4,500 rpm and subsequently, bacterial cell sediment was rinsed with distilled water. The bacterial concentration of the obtained suspension was justified at 10^9 (CFU/ml) by absorbance estimation at 600 nm (OD=1) using a spectrophotometer (Bahloul, 2013). The alga *S. platensis* was grown on Zarrouk medium (Zarrouk, 1966) and was incubated in growth chamber under continuous illumination (2000 lux) at 35˚C± 2˚C for 30 days. The inoculate suspension was approximately adjusted to 10^9 CFU/ml culture (colony formation unit). Trafose K (1) (Potassium phosphite (K_2O_2 8% + P_2O_5 42%) used rate at 2.5%. For preparing Salix water extract, dried leaves and bark of Salix plant was obtained from the Botanical garden, Agriculture Research Centre Giza Egypt. The dry leaves and bark of the Salix crushed into powder. 0.5 gm of the powder was put in 50ºC heated water and left for 1 hr, then filtered using filter paper No. 102 into a conical flask. An equivalent of 10 mg dried material per ml of aqueous infusion was obtained (Adebolu and Oladimeji, 2007).

***Biotic and abiotic study:-**

Biotic and abiotic elicitors (*Serratia marcescens*, *Spirulina platensis*, Trafose K (1) and Salix water extract were applied 7 days after inoculation with 3000 second stage juveniles of *Meloidogyne sp*. Five plastic bags with sour almond seedlings were inoculated with 3000 second stage juveniles of *Meloidogyne sp* and left untreated. Also five plastic pots with sour almond seedlings each left untreated and un-inoculated with nematode to serve as a control. Each treatment was replicated five times and all treatments were arranged in a complete randomized block design. Pots were kept in the greenhouse at 25 ± 5^0C, receiving water and ordinary nutrient solution as required. Sour almond plants were tested 90 days after nematode inoculation.

***Determination of Nematode and Plant parameters:-**

1- Nematode parameters:

Plants were carefully uprooted then the infected roots with *Meloidogyne sp* were rinsed gently with tap water to remove soil particles. Galls and egg mass were counted in one gm roots. The same weight of roots were stained with lactic acid fuchsin to examine the developmental stage and females (Byrd *et al* 1983). The second juvenile stage (J_2s) extracted from 250 gm soil using Bermann funnels technique Galls, egg mass, developmental stages were counted under steroscop, while J_2s were counted using counting slid using light microscop. Reduction percentages of all nematode parameters in all treatments were compared with plants infected with nematode only.

Plant parameters:-

A- Plant growth:-

Weights (gm) and heights of shoots and roots length (cm) for plants of each treatment and control were determined**.**

B- Chemical components:-

Determination of phenolic compounds from {shoot + leaves}) was carried out according to that method described by Daniel and George (1972). Contents of free proline from {shoot + leaves}) were determined according to Bates (1973). Catalase activity was assayed according to Chen *et al.*, (2000). The reaction mixture with final volume of 10 ml containing 40 µl enzyme extract was added to 9.96 ml H_2O_2 phosphate buffer pH 7.0 (0.16 ml of 30% H_2O_2 to 100 ml of 50 mM phosphate buffer). Catalase activity was determined by measuring the rate of change in H_2O_2 absorbance in 60 second with a UV- spectrophotometer (Jenway) at 250 nm. The blank sample was made by using buffer instead of enzyme extract. One unit of

enzyme activity was defined as the amount of the enzyme that reduced 50 % of the H_2O_2 in 60 second at 25^0C.

Peroxidase activity was assayed using solution containing 5.8 ml of 50 mM phosphate buffer pH 7.0, 0.2 ml of the enzyme extract and 2 ml of 20 m M H_2O_2 after addition of 2 ml of 20 mM pyrogallol, the rate of increase in absorbance as pyrogallol was determined spectrophotometrically by UV- spectrophotometer (Jenway) within 60 second at 470 nm and 25^0C (Srivastava ,1987).

Determination of endogenous hormone (salicylic acid) in leaves of the treated plants as well as the control was carried out as described by Lee *et al.*, (1989).

D- Statistical analyses.

Experimental data were subjected to one way analysis of variance (ANOVA) and the differences between means were separated using the (L.S.D) at 5% level of probability using M- state software (Snedecor and Cochran, 1982).

RESULTS AND DISCUSSION

A- The effect of gibberellic acid pre-treatment with stratification on sour almond seeds:-

Data presented in table (1) revealed that all used treatments significantly increased percentage of radicle protrusion of sour almond compared to the control seeds.

Gibberellic acid at 1000 ppm recorded the highest percentage of radicle protrusion values (83.1 & 83.37%) followed by GA_3 at 500, while control seeds occurred the least percentage of radicle protrusion (54.3 and 51.97 %) in the two seasons.

Germination is a very complex physiological process, controlled by a range of developmental and external cues. Genetic and physiological studies have shown the important role played by plant hormones in regulating seed germination. Many studies have shown that breaking dormancy by after-ripening stratification and darkness is strongly correlated with hormone- balance, a decrease of ABA and increase of GA_s in seeds (Gubler *et al.,* 2005).

Results in table (1) showed also differences in stratification period between treatments. Seed radical emergence percentages occurred earlier and was more uniform during the stratification of the treated seeds in comparison with the control ones. No significant differences in plant height between treatments under study were detected in the first season. However, in the second season, plant height of the control ones was less than that of the other treatment which was statically equal.

These results were parallel to those of Hartman *et al.,* (2011) on seeds of stone-fruit species. However, increasing seedling growth due to GA_3 may be due to that GA_3 causes a new production of α- amylase which converts starch to sugar and provide energy for growth. No significant difference in number of leaves between the treatments in the first season. But, in the second season, 1000 ppm concentration of GA_3 induced the highest significant number of leaves. There are three important actions of GA_3; the first action is that GA intensifies an organ ability to function as a nutrient sink. The second one is GA ability to increase the synthesis of IAA in plant tissues. The third action involves accelerated synthesis of hydrolytic enzymes (Addicott and Addicott, 1982).

Data of plant diameter (Table 1) showed that there are no significant differences between treatments under study in the first season. But in the second season GA_3 at 1000 ppm resulted in the highest significantly plant diameter.

Table 1. percentage of radicle protrusion; stratification period; plant height; leaves number and plant diameter as affected by seed treatments on sour almond rootstock in seasons (2014 and 2015).

Seed Treatment	season 2014					Season 2015				
	percentage of radicle protrusion	Stratification period (days)	Plant Height	leaves Number	Plant diameter	percentage of radicle protrusion	Stratification period (days)	Plant height	leaves Number	Plant diameter
H2O (control)	54.3 C	25 A	33.67 A	65.33 A	0.3 A	51.97 C	25 A	32.3 B	26.67 B	0.26 B
GA3(500 P.P.M)	64.03 B	20 B	42.01 A	61.67 A	0.3 A	63.1 B	19 B	38 A	22.6 B	0.33 AB
GA3 (1000 P.P.M)	83.1 A	10 C	44.67 A	57.0 A	0.36 A	83.37 A	11 C	40.3 A	45.3 A	0.4 A

Means on each column followed with the same letter(s) are not significant different at 5 % level.

B– The effect of biotic and abiotic compounds for induction of systemic resistance and increasing tolerance against infection with *Meloidogyne sp*:-

1- <u>Nematode parameters</u>:-

The obtained results in Table (2) showed that all treatments greatly suppressed the nematode juveniles in the soil and reduced number of root galls, females, egg masses and development stages per roots. The use of *S. marcescens* recorded 40 %, 58.3 % and 75 % reduction in numbers of *Meloidogyne sp* juveniles in the soil, numbers of galls and number of females in root, respectively in season 2014. These

values in 2015 were 55. 6 %, 75 % and 33.3 %, respectively. These results are in harmony with those obtained by Bahloul, (2013) who stated that the bacterial strain *S. marcescens* showed the highest protease, chitinase and gelatinase activities, which might help to explain the way how the bacteria could act against the root-knot nematodes. It should be stated that the highest nematicidal activity was exhibited by these strains against the second stage juveniles of *Meloidogyne incognita*. These strains proved to be the most efficient isolates as nematicidal agents.

Data showed that application of Trafose K (1) in season 2014 recorded 66.7 % and 25 % reduction of number of galls and females in root, respectively. On the other hand, these values in 2015 season reached 56.3% and 66.7 %, respectively.

In this respect, Olivieri *et al.,* (2012) referred that resistance inductors include phosphites and formulations based on plant extracts and by-products of plant agro industries. When applied to plant leaves, phosphites can control diseases by two separate mechanisms: by directly affecting the pathogen and/or by activating the plant defense responses.

Data showed that Salix extract in season 2014 recorded 80 % and 75 % reduction in numbers of *Meloidogyne sp* juveniles in soil and numbers of females in root, respectively. On the other hand, these values in 2015 season reached 55.6 and 66.7 %, respectively.

It is a hydroxyl group bearing phenolic compound. Phenolic compounds including SA play important roles in lignin biosynthesis, act as allelopathic compounds, and regulate plant responses to a biotic stimuli and pathogen attacks (Vlot *et al*., 2009)

Table 2. Effect of the biotic (*S. marcescens* and *S. platensis*) and abiotic (Trafose K1 and Salix extract) elicitors on the reproduction of *Meloidogyne sp*. Infecting sour almond seedling during seasons 2014 and 2015.

Treatment	Season 2014										Season 2015									
	juveniles in 250 g soil		galls in(1gm) root		females in(1gm) root		egg masses in (1gm) root		development stages(1gm)		juveniles in 250 g soil		galls in(1gm) root		females in(1gm) root		egg masses in (1gm) root		development stages(1gm)	
	Average No.	Reduction %	Average No.	Reduction %	Average No.	Reduction %	Average No.	Reduction %	Average No.	Reduction %	Average No.	Reduction %	Average No.	Reduction %	Average No.	Reduction %	Average No.	Reduction %	Average No.	Reduction %
S.marcescens	60.00 B	40	5.00 BC	58.3	1.00 B	75	1.00 C	50	2.00 BCD	60	40.00 B	55.6	4.00 D	75	2.00 AB	33.3	1.00 B	50	1.00 CD	75
S.platensis	100.0 A	00	7.00B	41.7	1.00 B	75	1.00 C	50	3.00 AC	40	40.00 B	55.6	6.00 CD	62.5	2.00 AB	33.3	0.00 B	50	2.00 BC	50
Trafose K1	20.00 C	80	4.00C	66.7	3.00 A	25	1.00 B	50	1.00 CD	80	20.00 C	77.8	7.00 C	56.3	1.00 BC	66.7	0.00 B	50	3.00 AB	25
Salix extract	20.00 C	80	11.00 A	8.3	1.00 B	75	1.00 C	50	4.00 AB	20	40.00 B	55.6	10.00 B	37.5	1.00 BC	66.7	1.00 A	50	3.00 AB	25
Nematode only	100.0 A	--	12.00 A	--	4.00 A	--	2.00 A	--	5.00 A	--	90.00 A	--	16.00 A	--	3.00 A	--	2.00 A	--	4.00 A	--

Means on each column followed with the same letter(s) are not significant different at 5 % level.

Data presented in fig (1) illustrate that using *S. marcescens*; *S.platensis*; Trafose K(1) and Salix extract has recorded 60 %, 40 %, 80 % and 20 % reduction in number of development stages, respectively in season 2014. Whereas, the corresponding values were 75%, 50%, 25% and 25% reduction of numbers of development stages respectively in season 2015. All treatment in seasons 2014 and 2015 recorded 50% reduction in numbers egg masses in root.

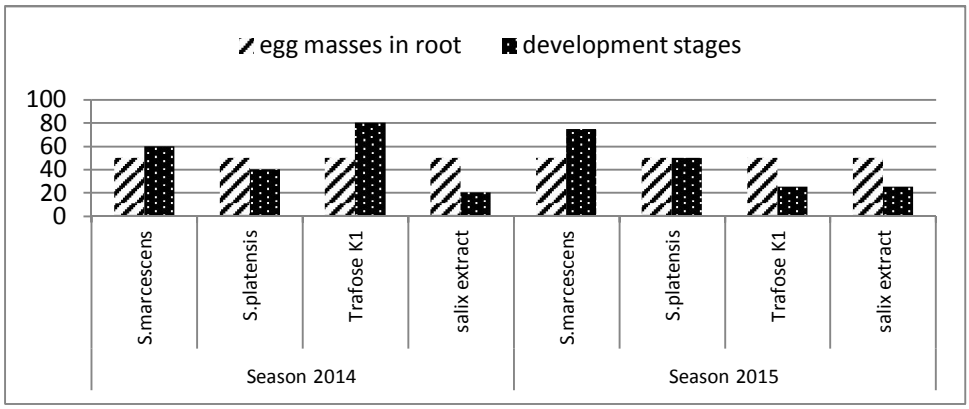

Fig 1. Effect of biotic and abiotic elicitors on reduction of egg masses and development stages of *Meloidogyne sp.* in root.

It could be concluded that, using biotic and abiotic elicitors reduced the levels of juveniles in soil; galls; females and egg masses in roots in Sour almond seedlings {table (2) and fig (1)}.

2- Plant parameters:-

A) - Plant growth:-

The obtained data in table (3) show that there were no significant differences in values of roots length and fresh weight in 2014 season while in 2015 plants treated with *S. marcescens* had the highest root length and fresh weight. Regarding the shoot height and fresh weight, there was no significant differences between treatments in shoot height in 2014 season, while, fresh weight and shoot height were the best with *S. platensis* treatment in 2014 and 2015 seasons.

These results agreed with Zeinat, *et al.*, (2009) who found that *Pseudomonas. fluorescens* and *S.marcescens* treatments significantly increased all growth parameters in the presence or absence of the pathogen and confirmed that *S. marcescens* and *P.fluorescens* were potent as bio-control agents for root-knot nematodes. Our results demonstrated that nematode challenged plants, as a result *S. platensis* inoculants showed reduced level of population of *Meloidogyne sp* infection.

Microalgal metabolites attracted attention, because they are sources for toxins, and potential new drugs (Shimizu 2003). Our result, showed that Salix extract increased plant growth in terms of shoot length, shoot fresh weight, root length and root fresh weight as compared to the inoculated untreated plants. Root galls, nematode population in root as well as in soil and root Pretreatment with Salix extract showed better plant growth and lesser intensity of root-knot disease as compared to the post-inoculation treatment. Similar results were obtained when the effect of SA was explored on *M. incognita* infesting of cowpea and okra (Nandi *et al*., 2003).

Table 3. Effect of the biotic (*S. marcescens* and *S. platensis*) and abiotic (Trafose K1 and Salix extract) elicitors on growth parameters of sour almond root stock infected with *Meloidogyne sp* during 2014 and 2015 seasons.

treatment	Season 2014				Season 2015			
	Root		Shoot		Root		Shoot	
	Length (cm)	Fresh weight(g)	height (cm)	Fresh weight (g)	Length (cm)	Fresh weight (g)	height (cm)	Fresh weight (g)
S.marcescens	19.67 A	2.933 A	39.00 A	3.100 BC	25.00 A	2.900AB	30.33 C	3.100 B
S.platensis	22.33 A	3.367 A	36.67 A	4.533 A	20.00 B	2.133 B	39.33 A	4.967 A
Trafose K 1	22.67 A	3.100 A	37.00 A	2.667 CD	19.33 B	2.633AB	35.67ABC	3.100 B
Salix extract	19.33 A	3.167 A	37.67 A	2.933 BC	19.00 B	3.633AB	37.00 AB	3.167 B
Nematode only	24.00 A	2.867 A	35.00 A	2.267 D	22.33 AB	3.733A	39.33 A	3.033 B
Control	19.00 A	2.000 A	37.00 A	3.533 B	24.33 A	2.200 B	33.33 BC	3.300 B

Means on each column followed with the same letter(s) are not significant different at 5 % level.

B) - Chemical components:-

Table (4) showed that root knot nematodes *Meloidogyne sp* caused significant increase in free proline contents, catalase peroxidase activities and salicylic acid in shoots of the infected plants compared with to control plants during 2014 season. During 2015 season this increase was only significant in case of catalase; peroxidase activity and salicylic acid content. Concerning the effect of tested elicitors on plants challenged plants with *Meloidogyne sp*, it was found that *S. marcescens*, trafose K(1) , *S. platensis* and Salix extract, respectively show significant increase in total phenols content of sour almond shoots when related to nematode only treatment, respectively. This was the case throughout the two seasons (2014-2015).

Data are shown in table (Table 4) quit on evidence that the greatest values of phenolic contents and Proline accumulation were achieved by using *S. marcescens* inoculation on the nematodes infected plants followed by Salix extract, Trafose K1

and *S. platensis*, respectively more than on the control plants, indicating the induction of systemic acquired resistant (SAR). These results are in agreed with Akram *et al*. (2013) who found that a significant increase in total phenolic contents was observed in bacterial-treated plants. Proline accumulation is a common metabolic response to both abiotic and biotic stress and when higher plants are exposed to stress; many plants accumulate high amounts of proline in thier tissues. In our experiment, a higher amount of proline in infected plants was observed.

Table 4**.** Effect of the biotic (*S. marcescens* and *S. platensis*) and abiotic (Trafose K1 and Salix extract) elicitors on total phenols, free proline contents, catalase; peroxidase activities and salicylic acid content in sour almond un-infected and infected with root-Knot nematodes during 2014 and 2015 seasons.

Treatment	Season 2014					Season 2015				
	Phenolic mg/100g D. W	Proline mg/100g D. W.	Catalase Ug/g F. W.	Peroxioxidase U g/g F.W.	SA (mg/100g F. W.)	Phenolic mg/100g D. W.	Proline mg/100g D. W.	Catalase Ug/g F. W.	Peroxioxidase g/g F.W.	SA (mg/100g F. wt.)
S.marcescens	0.54 A	0.727 A	2.120 A	2.467 A	1.267 A	0.417 A	0.83 A	1.733 A	1.303 A	1.480 A
S.platensis	0.33 B	0.1073D	0.872 C	0.713 D	1.041 B	0.332BC	0.124 D	0.996 C	0.601 D	0.803 D
Trafose K1	0.36 B	0.134 D	0.799 C	0.656 D	0.701 C	0.406AB	0.578 B	0.871 CD	1.204 B	0.969 C
salix extract	0.22 C	0.492 B	1.947 B	1.373 B	0.970 B	0.253 C	0.381 C	1.172 B	0.7637C	1.253 B
Nematode only	0.099 D	0.352 C	1.867 B	0.868 C	0.681 C	0.107 D	0.174 D	1.157 B	0.717 C	0.913 C
control	0.084D	0.117 D	0.877 C	0.348 E	0.278 D	0.120 D	0.129 D	0.7667D	0.301 E	0.611 E

Means on each column followed with the same letter(s) are not significant different at 5 % level.

As for Catalase and Peroxioxidase activities, data showed an increase in their activities in some treatments in relation to controls (Table 4). It is quite evidence that, the greatest activities were achieved by using *S. marcescens* inoculation on the nematode infected plants, followed by Salix extract and *S. platensis*, more than that in the healthy plants, indicating the induction of systemic resistant (SR). This was the case throughout the two seasons 2014 and 2015. Plants have endogenous defense mechanisms that can be induced in response to attack by, plant parasitic nematodes. It is well known that the defense genes are inducible genes and appropriate stimuli or signals are needed to activate them. Inducing the plant's own defense mechanisms by

prior application of a biological inducer is thought to be a novel plant protection strategy (Ramamoorthy *et al*., 2001). In the present study, increased total phenol, free proline and defense enzyme such as catalase and peroxidase activities were observed in infected plants. It was noticed that in challenged plants with root knot nematodes (*Meloidogyne sp*), plant treatment with *S. marcescens* resulted in increasing the contents of SA compared with those of nematode infected plants. In challenged treatments, significantly higher SA levels were observed in leaves of plants treated with *S. marcescens* in comparison with plants treated with *S. platensis;* Trafose K (1) and salix extract respectively (Table 4). Our results demonstrated that, in challenged plants, treatment with *S. marcescens*, *S. platensis* as well as Salix extract resulted in increasing the contents of SA compared with those of nematodes infected plants. In this respect (Ueno *et al*. 2011) reported that exogenous application of SA and IAA has been demonstrated to enhance plant resistance against pathogens by acting as potent inducer of systemic resistance.

CONCLUSION

Using GA_3 at 1000 ppm before stratification is necessary for breaking dormancy and enhancing radicle protrusion of sour almond rootstock seeds. Using *(Serratia marcescens*, *Spirulina platensis*, as biotic inducers or *Salix alba* extracts as natural inducers as soil treatments increased the induction of systemic acquired resistance (SAR) against to root knot- nematode infected sour almond rootstock and show good systemic acquired resistance in controlling nematode infection.

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كسر سكون بذور اللوز المر وتقييم بعض المستحثات الحيوية واللاحيوية ضد الإصابه بنيماتودا تعقد الجذور (*Meloidogyne sp.*) فى الشتلات المنتجة

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تم دراسة تأثير إستخدام تركيزات مختلفة من حمض الجبريليك على تلسين بذور اللوز المر وفترة التنضيد اللازمـــة وإرتفاع النبات وعدد الأوراق وقطر النبات وذلك خلال موسمي 2014 ، 0 2015

وفي تجربه أخري أجريت في الصوبه إستخدمت (*Serratia marcescens* ' *Spirulina platensis*) {كمستحثات حيويـــة} ، ترافوس (1) k ومستخلص نبات الصفصاف Salix extract {كمستحثات لا حيويـــة} تضاف للتربه لمعرفة تأثيرها علي إستحثات المقاومه الجهازيه لأصل اللوز المر المصاب بنيماتودا تعقد الجذور وعلاقة ذلك بتكاثر النيماتودا من حيث عدد اليرقات في التربـــة وعدد العقد الجدريـــة وعدد الإناث وأكياس البيض وتأثير ذلك علي الصفات الخضريـــة مثل طول ووزن المجموع الخضري والجذري0

وأيضا تم دراسة بعض الصفات الفسيولوجيـــة داخل النبات. حيث تم تقدير محتوى الفينولات والبرولين وحمض السالسيلك ونشاط كلا من إنزيم الكتاليز وإنزيم البيروكسيديز.

أظهرت النتائج أن :

- التركيز 1000 جزء في المليون من حمض الجبريليك كان له تأثير علي نسبة التلسين للبذور مفارنة بباقي المعاملات. حيث حقق نسبة تلسين 1ر83% .
- أظهرت البيانات أن إستخدام *Serratia marcescens* سجلت إنخفاض قدره 40% ، 3ر58% ، 75% في أعداد يرقات *Meloidogyne sp.* في التربة وعدد البيض وعدد الإناث في جذور النبات علي الترتيب في سنة 2014 – بينما وصل الانخفاض الناشيء في هذه الصفات نتيجة المعامله بـــــ *Spirulina platensis* 6ر55% ، 5ر62% ، 3ر33% في أعداد *Meloidogyne spp.* في سنة 2015.
- إستخدام *Spirulina platensis*, *Serratia marcescens* . Trafose k(1) ومستخلص الصفصاف سجلت 60% ، 40% ، 80% ، 20% في عدد الأطوار الكامله من النيماتودا لسنة 2014. بينما سجلت هذه المعاملات إنخفاض في عدد الأطوار الكامله قدره 75% ، 50% ،25% ،25% في الأطوار الكاملة من النيماتودا لسنة 2015.

- إستخدام *Serratia marcescens* ومستخلص نبات الصفصاف حسن من صحة النبات عن طريق زيادة النمو الخضري بما يعادل الكنترول .
- التأثير الايجابي للمعاملات المستخدمه لم يتضح فقط في زيادة الفينولات الكليه والبرولين الحر ، لكن إيضا كان واضحا فى زيادة نشاط إنزيم الكتاليز والبيروكسيديز بالمقارنة بالكنترول0
- كل المستحثات المختبرة أدت الي زيادة معنويه في مستوي حمض السالسيلك عند مقارنتها بالكنترول.

ويمكن التوصية بالأتي :-

- إستخدام حمض الجبريليك بتركيز 1000 جزء في المليون قبل الكمر البارد ضروري لكسر سكون البذور والإسراع من عملية تلسين بذور أصل اللوز المر .
- إستخدام *Serratia marcescens* , *pirulina platensis* كمستحث حيوي ومستخلص نبات الصفصاف ومركب Trafose k(1) كمستحث غير حيوي كإضافة في التربة تعتبر معاملات واعدة وتؤدي إلي إستحثاث مناعة داخلية ضد الإصابة بالينماتودا.