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Inducing systemic acquired resistance (SAR) against root-knot nematode *Meloidogyne javanica* and evaluation of biochemical changes in cucumber root

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Summary

For inducing systemic acquired resistance (SAR), Salicylic acid (SA), Ascorbic acid (AA), and silicon (Si) were applied on shoots, roots, and both of them simultaneously. Results showed that all treatments reduced the number of galls, root gall index, number of egg mass/root system, number of nematodes/root system, number of eggs/root system, number of nematodes/pot soil, the final population density of nematodes, and rate of reproduction. Treatments also increased growth criteria, including chlorophyll, shoot fresh weight, root fresh weight, shoots dry weight, root dry weight, shoots length, and root length. SA foliar and root application decreased infection criteria and increased total phenol, peroxidase, and phenol oxidase activities. Ascorbic acid and silicon increased total phenol, peroxidase, and phenol oxidase activities.
Keywords: *M. javanica;* SAR; Salicylic acid; ascorbic acid; Si; polyphenol oxidase; peroxidase; total phenol

Introduction

Plant parasitic nematodes are responsible for severe yield loss, reduced crop production, as well as cause damage to economically important crops (Schleker *et al.,* 2022). Root-knot nematodes are one of the most important groups of plant parasitic nematodes and cause heavy yield losses annually, causing significant damage and production loss in many cultivated plants, particularly vegetable crops (Feyisa, 2021). Root galls' induction has been shown to restrict water and nutrient uptake, leading to wilting, mineral deficiencies, reduced plant growth, and decreased yield and plant biomass (Hallmann, 2018). *In addition, Meloidogyne* spp. is one of the most destructive pathogens in cucumber crops which causes damage and reduces growth and yield loss (Muther, 2020); the yield loss may reach up to 50 % (Katooli *et al.,* 2010).

During a survey carried out in north Iraq, results showed that a high disease incidence was in cucumbers 58 % and tomatoes 33 %, which are considered the most common vegetable grown under greenhouses (Hamad *et al.,* 2022). Surveys in Erbil province showed a wide distribution of *M. javanica* in cucumber fields Ami, (2010). There are also studies in Iraq to use chemicals and plant extract to control root-knot nematodes Taher (2019).

Systemic acquired resistance induced in tomatoes against root-knot nematode reduced infection criteria and improved growth indices Bakr and Hewedy (2018). Similarly, the application of SA increased some defense compounds, such as hydrogen peroxide and peroxidase activities in the root of cucumbers infected by *M. javanica* (Siahpoush *et al.,* 2011). Treatment with ascorbic acid

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significantly enhanced plant growth parameters, including shoot and root weight and length. Moreover, compared with untreated control plants, these resistance inducers enhanced the synthesis and activity of defense enzymes, peroxidase, and phenol oxidase. Ziadi *et al.* (2001).

Recent investigations have shown a reduction in the nematode population of the root-knot nematodes lower number of eggs, juveniles, and galls in the presence of silicates Roldi *et al.* (2017). Additional studies illustrate that silicon lowers the effect of nematode on cucumber and many other vegetable crops by decreasing infection parameters Kaushik and Saini (2019).

This investigation expands upon previous studies to show: 1) the detection of inducing systemic acquired resistance (SAR) in plants by foliar applications of salicylic acid, ascorbic acid, and silicon, 2) the evaluation of SAR criteria, including peroxidase and polyphenol oxidase activities, and 3) assessment of plant vigor through growth and infection measurements.

Material and Methods

M. javanica pure culture:

Three-week-old tomato (*Solanum lycopersicon* cv. Pomodoro-Marglobe) seedlings grown in plastic trays filled with sterilized peat moss and soil at the rate of 1:3 and then infected with egg masses of identified species of *Meloidogyne* spp. and maintained in the greenhouse after 5 – 6 weeks; plants infected with the preparation of nematode egg mass suspension.

Extraction of Root-knot nematode eggs

The root of infected tomato seedlings from pure culture was brought to the plant pathology laboratory - Plant Protection Department for the extraction of eggs according to the method described by Coyne *et al.* (2007). Tomato root from pure culture was washed and cleaned up, then cut into small pieces 1 – 2 cm and moved to a beaker containing 0.5 % sodium hypochlorite (NaOCl). Then put in the blender and blend for one minute, Subsequently poured through 250 µm, 75 µm, and 25 µm sieves to collect eggs on the 25 µm sieve.

Preparation of Cucumber Seedlings and soil infestation

Seeds of the most common hybrid cucumber cultivar (Super Faris F1) were sowed in a black plastic tray with peat moss. Three cucumber seedlings were transplanted into pots 30 cm in diameter containing sterilized soil when they reached three true leaves and were infested with 1000 nematode eggs/Pots.

Inducing Systemic Acquired Resistance (SAR)

The following substances were used to induce Systemic Acquired Resistance (SAR) in cucumber plants against root-knot nematode *M. javanica*:

Salicylic acid (SA) and Ascorbic acid (AS) at a concentration of (50 mM). SA dissolved in ethanol and then diluted with distilled water to the appropriate concentration. The barrier formula from Cosmocel Company is used as a source of Silicon (Si) and used according to the rate of application mentioned by the produced company, 0.03 ml/liter. Each substance was applied on cucumber plants by three methods: first treatment of leaves and roots application, second applied only on roots, and third applied on foliar parts. Each treatment was replicated three times, once after transplanting seedlings to pots and after 15 and 30 days. After two months of infestation of soil by *M. javanica* following characteristics were calculated:

Chemical criteria

The Following chemicals were estimated in cucumber roots to determine the resistance and defense reflection of cucumber plants:

<u>*Estimation of total phenolic substances.*</u> The infected roots sample of 0.5 g for each sample root-knot was crushed and ground with a pestle and mortar in 10 ml of 80 % ethanol, then centrifuged at 5000 rpm for 20 minutes. The supernatant was pooled and evaporated for drying and removing the ethanol remain. The residue was dissolved in 10 ml of distilled water, and 0.5 ml of folin-ciocalteu reagent was added. The latter was left for 3 minutes and added 2 ml of 20 % Na2CO3 was. The solution was mixed thoroughly for 1 minute, placed in boiling water, and left to cool down.

Absorbance measurement was done at 650 nm in a colorimeter and compared with a blank. The total phenol concentration in test samples was calculated by comparing with the standard curve and expressed as mg/g material (catechol) Nayak, (2015).

<u>*Estimation of peroxidase activity*</u> The activity of peroxidase was determined according to a standard procedure where 0.5g of the root was homogenized in two ml of 0.1 M sodium phosphate buffer (pH 6.5) and centrifuged at 10,000 rpm for 15 min at 4 °C. Supernatant was used as an enzyme source where. 100 ml of enzyme extract was mixed with 1.5 ml of guaiacol (0.05 M). 100 ml of hydrogen peroxide (1 %) (v/v) was added to initiate the reaction. The absorbance was read at 420 nm. Sharma and Sharma (2017).

<u>*Estimation of polyphenol oxidase activity*</u> Root in weight of 0.5g was homogenized in 2 ml of 0.1 M sodium phosphate buffer (pH 6.5), then centrifuged at 16,000 g for 15 min at a temperature of 4 °C. The supernatant was used as the enzyme source. The reaction mixture consisted of 200 µl of the enzyme extract and 1.5 ml of 0.1 M sodium phosphate buffer (pH 6.5). Catechol was added to start the reaction. 200 µl of 0.01 M catechol is added, and the activity is expressed as changes in absorbance (Sahebani *et al.,* 2011.). All experiments were designed as randomized complete block designs (RCBD) in three blocks under the greenhouse, and each block included experimental units distributed randomly.

The comparison between means was carried out according to Duncan's multiple range test (Duncan, 1951) ($P < 0.05$) using a computerized program of SAS (2001).

Infection criteria

The number of root-knot (root galls) The number of galls on the cucumber root system was calculated for each root system of cucumber plants (each replication) after washing and cleaning the roots from remain soil Galling index (GI).

Galling index (GI) The root gall index was calculated according to a scale by Taylor and Sasser (1978) (Kumar *et al.,* 2010). They rated the index 0 to 5 scale as follows: 0 = no gall on the root, 1 = 1–2 galls, 2 = 3–10, 3 = 11–30, 4 = 31–100, and 5 = more than 100 galls/root system.

The number of the egg mass Number of egg mass calculated after Egg-mass staining, *Meloidogyne spp.* egg masses on the root surfaces were stained bright red after washing the root and submersion it in a 20 % red food color solution for 15 minutes. The root was washed with tap water, and egg mass was counted if visible by naked eyes or under a stereomicroscope (Thies *et al.,*2002)**.**

The number of nematode stages/roots system Nematodes stained by the method mentioned above and stained nematode by red food color number of nematode stages/root system calculated inside roots under a stereomicroscope,

The nematode population density in soil The soil of each pot brought to the laboratory and population density were calculated after the nematode extraction by the Try method (Coyne, 2007).

Increment percentage of nematode population in the soil Increasing nematode population in soil calculated according to the following equation:

Increment percentage of nematode population (%) = Final population of nematode - initial nematode population **/** Initial nematode population *100

Rate of nematode reproduction The rate of nematode reproduction is calculated according to the following equation:

Rate of nematode reproduction (%) = Final nematode population / Initial nematode population* 100

- Final nematode population= Number of nematodes in soil +number of nematodes in roots +number of eggs in the root.

Percentage of reduction The rate of reduction in each infection criterion is measured according to the following equation:

Percentage of reduction (%) = Control (infested soil) – treatment/ Control (infested soil) *100

Plant growth criteria

Chlorophyll Chlorophyll (SPAD) is measured using a chlorophyll meter (SPAD502/Konica Minolta Sensing, INC., made in Japan).

Improving the percentage of growth criteria Improving the percentage of each growth criterion calculated according to this equation:

Percentage of improvement (%) =Treatment – Control (infested soil) / Control (infested soil) *100

Table 1. Effect of salicylic acid, ascorbic acid and (Silicon + Calcium) application on growth criteria of Cucumber infected by *Meloidogyne javanica.*

	Growth Criteria						
Treatments	**Chlorophyll (Spad)**	**Shoots fresh weight (g)**	**Root fresh weight (g)**	**Shoots Dry weight (g)**	**Root Dry weight (g)**	**Shoots length (cm)**	**Root length (cm)**
Healthy plant	17.17 a	61.13 a	17.17 a	10.787 a	1.089 a	71.3 a	32.37 a
salicylic acid (R+F)	14.97 b	53.7 b	14.97 b	9.373 b	0.9507 b	63.37 b	23.9 b
salicylic acid (R)	14.03 c	51.8 bc	14.03 c	9.03 bc	0.8627 c	61.3 b	21.47 c
salicylic acid (F)	13.6 c	50.97 bcd	13.6 c	8.857 bcd	0.8313 cd	57.57 c	21.03 c
Silicon (R+F)	12.9 d	48.3 cde	12.9 d	8.333 cde	0.81 de	56.2 cd	19.9 d
Silicon (R)	12.2 e	47.67 cde	12.2 e	8.153 de	0.7663 ef	55.57 cd	19.63 d
Ascorbic acid (R+F)	12.17 e	46.7 de	12.17 e	8.03 e	0.759 f	53.2 de	19.63 d
Ascorbic acid (R)	12.13 e	46.5 de	12.13 e	8.007 e	0.7413 f	53.17 de	19.23 d
Silicon (F)	11.53 ef	46.37 e	11.53 ef	7.99 e	0.7157 fg	52.9 de	19 de
Ascorbic acid (F)	11.1 f	45.83 e	11.1 f	7.983 e	0.6857 g	50.07 e	18.87 de
Control (Infested, non-treated)	9.3 g	41.03 f	9.3 g	7.017 f	0.5837 h	44.9 f	18.07 e

* (R) =Root application, (F) = Foliar application, (R+F) = Root+ Foliar application. Each number is means of three replications.

* Means followed by different letters significantly different according to Duncan's multiple range tests, P ≤ 0.05

Shoot and root length (cm/plant) The lengths of shoots (cm/plant) were measured from the soil line to their terminal tip-off plant. Root length was measured after carefully pulling plants from the soil to avoid remaining root parts, then washed to remove soil, and another debris root length was measured from the crown region to the tip-off root by a ruler.

Fresh weight of roots and shoots (gm/plant) Roots were cleaned and washed, left in the laboratory to allow excessive water used for cleaning to evaporate, then weighed by the electronic scale. The electronic scale also weights the shoot system for each replication.

The dry weight of Roots and shoots (gm/plant) The shoots and roots of each treatment were cleaned and put on A3 or A4 paper envelopes according to the size of the shoots and roots and then placed in the oven at 70 °C for 48 hrs. Subsequently, put on benches in the laboratory for 15 minutes to allow any excessive vapor to get out from the bag. Dry weight was calculated using an electronic balance.

Results

As demonstrated in Table 1, results show the effect of salicylic acid, ascorbic acid, and silicon application on the growth criteria of cucumbers infected by *M. javanica*. Chlorophyll in the healthy plant was 17.17 followed by foliar and root application of salicylic acid was recorded at 14.97, which was a significantly better treatment for increasing chlorophyll percentage when compared with other treatments. The lowest rate was in infested (control) treatment 9.3 due to infection with M. javanica and without any treatment. Nematode infection affected the cucumber plant and led to weakness and less chlorophyll density in the leaves. Foliar application of salicylic acid was recorded at 13.6 followed by foliar and root application of silicon at 12.9, and root application of silicon at 12.2. Meanwhile, foliar and root application of Ascorbic acid was at 12.17. All treatments showed better chlorophyll content than infested control one.

The highest shoots fresh weight after healthy plants were foliar and root application of salicylic acid 53.7 g, followed by root application of salicylic acid 51.8 g, foliar application of salicylic acid (SA) 50.97 g, root and foliar application of silicon (Si) 48.3 g, root application of SC47.67g, root and foliar application of ascorbic acid (AA) 46.7 g then followed by other treatments and less than 9.3 g in control.

Root fresh weight in the healthy plant was 17.17 g, significant compared with infected control and other treatments which is normal results, since root-knot nematode causes damage to the roots and malformation in the root system, affecting infected cucumber plants. Root fresh weight in treatment with root and foliar application of SA was significantly better than in other 14.97 g, followed by the SA root application 14.03 g and SA foliar application 13.6 g. Shoots dry weight values were near the healthy plant in root and foliar application of SA 9.373 g followed by 9.03, 8.857, 8.153 and 8.03 g in SA root application, the SA foliar application, the Si root and foliar application, the Si root application, and then AA root and foliar application. Due to the high level of water contained in plant vegetative parts, the dry weight is a good way to estimate plant growth and is more reliable.

Root dry weight in the healthy plant was 1.089 g, followed by the

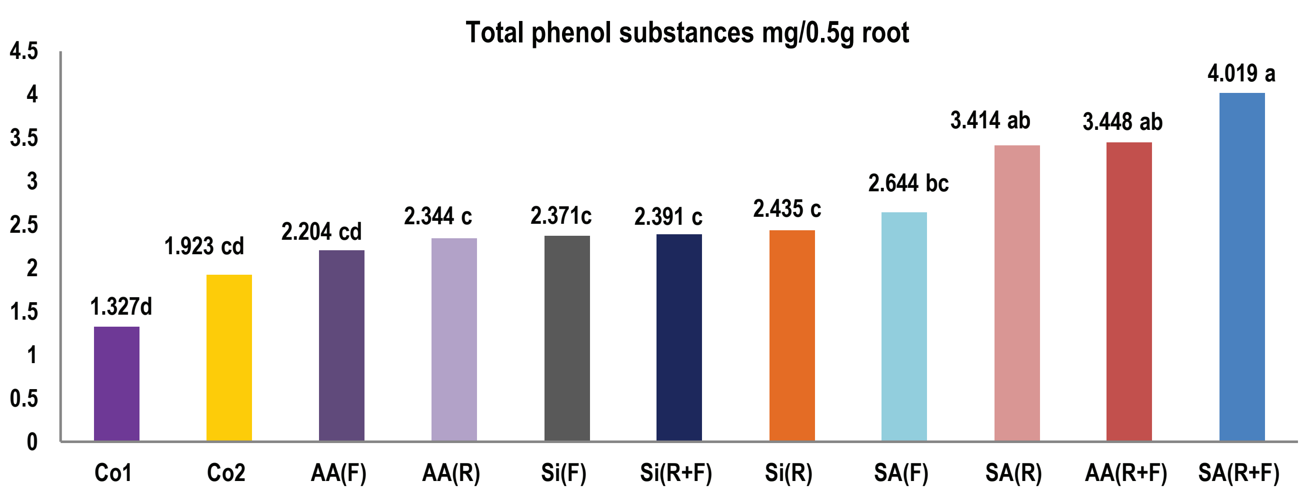

Fig. 1. Effect of salicylic acid, ascorbic acid, and Silicon application on total phenolic substances (mg/0.5g root) in Cucumber root infected by *Meloidogyne javanica.*
* Co1 = healthy plant, Co2 = control, (R) = root application, (R+F) = root and foliar application, AA = ascorbic acid. Si = silicon and SA = salicylic acid.
*Each number is means of three replications.
* Means followed by different letter(s) differ significantly according Duncan's multiple range test, P ≤ 0.05.

SA root and foliar application 0.9507 g, the SA root application 0.8627 g, the foliar application 0.8313 g, and the Si root and foliar application 0.81 g. After the SA treatments, the Si application recorded better results than AA. Less value, 0.5837 recorded in control without any treatments *M. javanica* affected in malformation and growth of roots.

Shoots length also recorded an improvement in SA application compared with control treatments; SA root and foliar recorded 63.37 cm length, SA adding to root zoon 61.3 cm shoot length. Si and AA applications also have a reasonable effect in shoot length compared to the control.

Root length in all treatments was shorter than in healthy plants due to root-knot infection, which reduces the host's root system. Foliar and root application of SA root length were 23.9 cm, significantly different and better than in the other treatments, followed by SA root application at 21.47 cm and SA foliar sprayed at 21.03 cm. The other treatments were not significantly different but still recorded better root length compared with the control 18.07 cm. Among the other treatments, Si root and foliar application were better, with a 19.9 cm root length. AA foliar spray has less effect in improving the root length.

Results of infection criteria, as illustrated in (Table 2) showed that the number of root Knot (galls) was less in SA root and foliar applications, 23 galls/root system compared with controls 37.33 galls/root system. Thus salicylic acid root and foliar application decreased the number of galls /root systems. The Si root and foliar application were at 25.67 gall/root system, followed by the SA root application and SA foliar application at 26 and 28.33, respectively. Ascorbic acid root application was recorded at 29. AA roots and foliar application at 30.67 gall/root system reduced gall number / root compared with control. Due to the wide range of the root gall index, the number is close, but they are still significantly different treatments than the control. The root-knot index was 3 in all of the following treatments: root and leaf application of SA, SA root application, SA leaf application, Si root and leaf application, Si root application, and AA root and leaf application. The leaf applications of AA and Si did not differ significantly from the control. The gall index was 4.

The number of egg mass in SA root and foliar application was 10.33. It was followed by Si root and leaf application 13, SA root application 15.33, SA leaf application 16.33, and AA root application 17.33. The AA root and foliar application added 17.67 Si to the root zone, AA foliar spray 18.33, and Si foliar spray 20.0. All treatments were recorded less egg mass/root system and significantly different from the control 26 egg mass /root system.

More nematodes/root systems were recorded in control 499.3 and less in SA root and foliar application 395.7 after healthy plant, which is zero (not infected). In the root and leaf application of Si Number of nematodes/root system was 404.3, then 415, 418.3, 418.7, 419.7, 443.7, 461.7, and 468 for each of the SA root application, SA foliar application, AA root application, AA root, and foliar application, Si root application, AA foliar and Si foliar application respectively.

All treatments reduced the number of eggs/Root system compared with the control 5984 eggs/root system. 2196 eggs/root system for SA root and foliar application, Si root and foliar application were at 2765, SA root application at 3262, SA foliar spray at 3521, and the AA root application was at 3651. The AA root and foliar application were at 3693, the Si root application at 3702, AA foliar application at 3935, and Si foliar application at 4258. The number of nema-

Table 2. Influence of salicylic acid, ascorbic acid, and Silicon application on infection criteria of Cucumber infected by *Meloidogyne javanica.*

	Infection Criteria							
Treatments	**No. of root knot (galls)**	**Root gall index**	**No. of egg mass/root system**	**No. of nematodes/root system**	**No. of eggs/root system**	**No. of nematodes / pots soil**	**Final population density of nematodes**	**Rate of reproduction**
Healthy plant	0.00 a	0.000 a	0.00 a	0.0 a	0 a	0.00 a	0.000 a	0.000 a
salicylic acid (R+F)	23.00 b	3.000 b	10.33 b	395.7 b	2196b	477.0 b	3068 b	3.068 b
Silicon (R+F)	25.67 bc	3.000 bc	13.00 bc	404.3 bc	2765bc	584.3 c	3230 bc	3.230 bc
salicylic acid (R)	26.00 bc	3.000 bc	15.33 cd	415.0 bc	3262cd	711.7 d	3754 bcd	3.754 bcd
salicylic acid (F)	28.33 cd	3.000 bc	16.33 cde	418.3 bc	3521cde	721.3 d	4418 bcde	4.418 bcde
Ascorbic acid (R)	29.00 cd	3.000 bc	17.33 de	418.7 bc	3651de	772.7 e	4839 de	4.839 de
Ascorbic acid (R+F)	30.67 cd	3.000 bc	17.33 de	419.7 bc	3693de	789.3 e	4842 cde	4.842 cde
Silicon (R)	30.67 cd	3.333 b	17.67 de	443.7 cd	3702de	841.0 f	4953 de	4.953 de
Ascorbic acid (F)	33.33 de	4.000 d	18.33 de	461.7 de	3935de	848.7 f	5252 de	5.252 de
Silicon (F)	33.33 de	4.000 d	20.00 e	468.0 de	4258e	859.7 f	5573 e	5.573 e
Control (Infested, non-treated)	37.33 e	4.000 d	26.00 f	499.3 e	5984f	874.7 f	7492 f	7.492 f

* (R) =Root application, (F) = Foliar application, (R+F) = Root+ Foliar application. Each number is means of three replications

* Means followed by different letters significantly different according to Duncan's multiple range tests, $P \leq 0.05$.

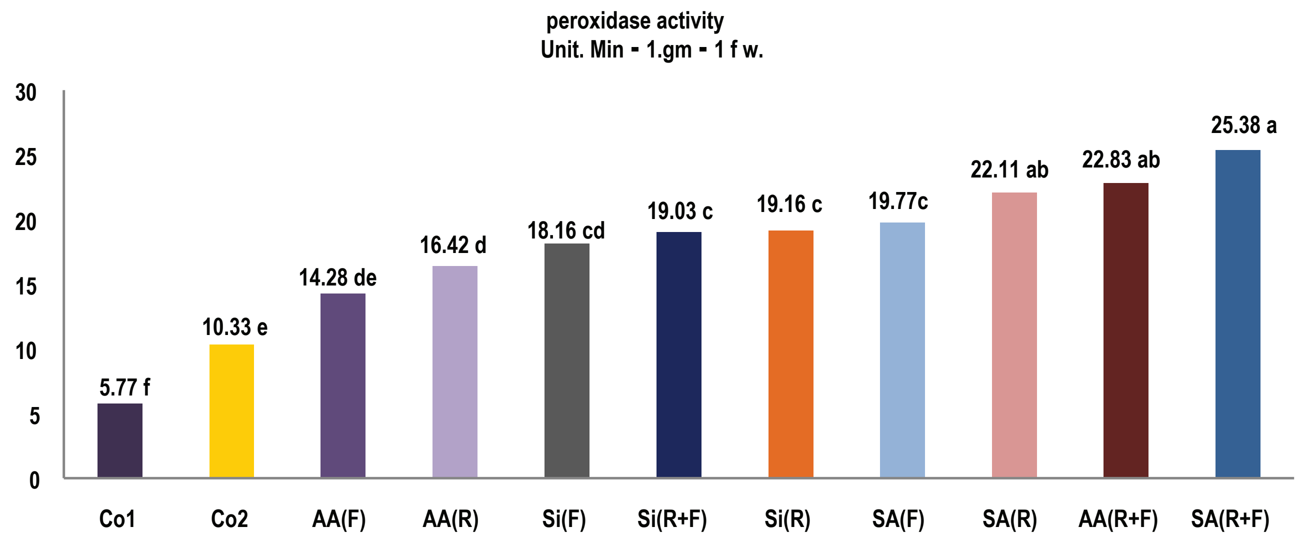

Fig. 2. Effect of salicylic acid, ascorbic acid, and Silicon application on peroxidase activity (Unit. Min-1.gm-1 f w.) in Cucumber root infected by *Meloidogyne javanica.*
* Co1 = healthy plant, Co2 = control, (R) = root application, (R+F) = root and foliar application, AA = ascorbic acid. Si = silicon and SA = salicylic acid.
*Each number is means of three replications.
* Means followed by different letter(s) differ significantly according Duncan's multiple range test, P ≤ 0.05

todes/pots soil in treatments used SA in both roots, and foliar part was at 477.0. A significant decrease in the number of nematodes compared with other treatments and control after the root and foliar application of Si with 584.3 nematodes/pots soil.

Salicylic acid root and SA foliar application were 711.7 and 721.3, respectively. Nematodes/soil pots were 789.3 for AA adding to root zoon 772.7, then root and foliar application with 789.3 nematodes/pots soil. No significant differences between root applications of Si 841.0, AA foliar spray 848.7 and Si foliar spray 859.7 were found, but still reducing the number of nematodes/soil pots compared with control recorded 874.7 nematodes/pots soil. The final population densities of nematodes were close. Still, all treatments declined the final number of nematodes and reduced the effect of nematodes on cucumber root 7492 in control. A smaller final population density of root-knot nematode was recorded in the treatment with Salicylic acid root and foliar application (3068 Si root followed by 3230 foliar application).

Salicylic acid added to root zoon resulted in 3754 nematodes. SA foliar sprayed yielded 4418 nematodes, 4839 nematodes for AA root application, and 4842 for AA root and foliar application. A lesser reduction was observed with Si treatments root application; AA foliar sprayed and Si foliar sprayed 4root zoon (953, 5252, and 5573).

All treatments in this experiment significantly reduced the reproduction rate in root-knot nematodes M. javanica which is so important for decreasing the damage and effect of nematodes and reducing population densities in the soil so that the next infection will be less severe on plants. The reproduction rate in control was 7.492, and less reproduction rate resulted from foliar root application of SA (3.068), then Si root and foliar application (3.230), SA added to root zone 3.754. The foliar spray of SA also increased the resistance of the cucumber plant. The reproduction rate reduced to 4.418, then to 4.839 and 4.842 for AA root application, followed by AA foliar and root application. All infection criteria adding AA in the root zone are more effective than application for both root and foliar for Si root application (4.953), AA foliar applications (5.252), and Si foliar sprays (5.252).

Results of total phenolic substances in cucumber plant roots (Fig. 1) showed that total phenol in root of healthy plant was 1.327mg and control recorded more phenolic substances 1.923mg as cucumber root react against pathogen root-knot nematodes and all other treatment increased number of phenolic substances as defense substances.

SA(R+F) recorded highest level of phenolic substances 4.019 mg and had significant effect on increments of total phenolic substances in root, then 3.44mg and 3.414 in AA(R+F) and SA(R) respectively.

Cucumber plant sprayed with SA recorded 2.644 mg and 2.435 mg for Si(R), 2.391 mg for Si(R+F), Si (F) 2.371 mg, AA(R) 2.344 and 2.204 mg for AA (F). Ascorbic acid applications were less effect treatments on phenolic substances increment on cucumber roots.

Effect of treatments salicylic acid, ascorbic acid, and Silicon application on peroxidase activity (Unit. Min-1.gm-1 fw.) in cucumber root demonstrated in (Fig. 2) due to the infection by *M. javanica* peroxidase activity pile up from 5.77 in healthy plant to 10.33 in control and reached the pick in SA(R+F) treatment 25.38 then 22.83, 22.11 in AA(R+F) and SA(R) they are significantly different from other treatment. SA (F), Si(R), Si(R+F), Si (F), AA(R) and AA

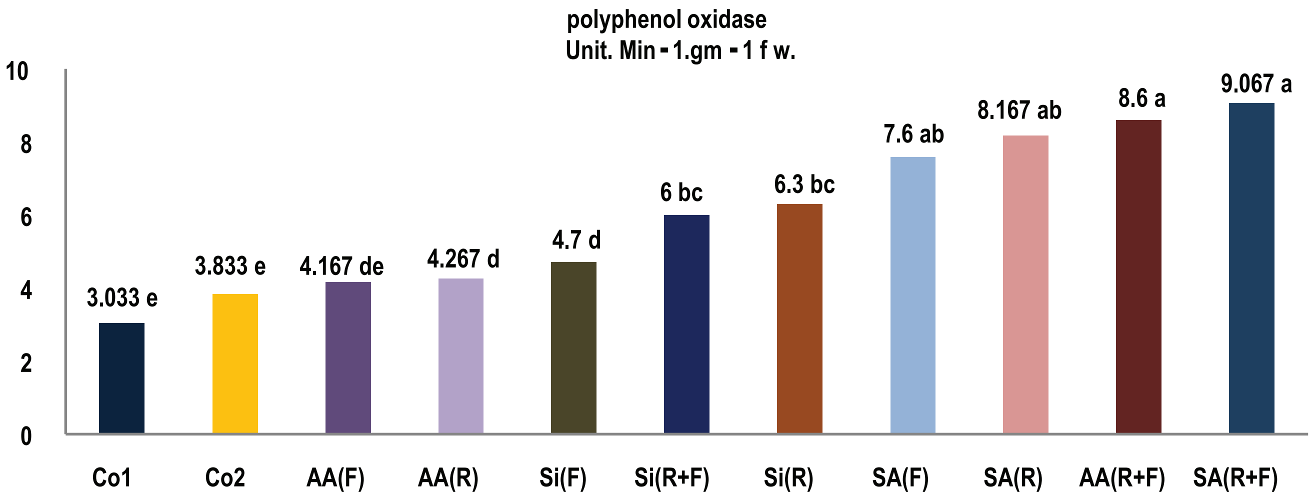

Fig. 3. Effect of salicylic acid, ascorbic acid, and Silicon application on polyphenol oxidase activity (Unit. Min-1.gm-1 f w.) in Cucumber root infected by *Meloidogyne javanica.*

* Co1 = healthy plant, Co2 = control, (R) = root application, (R+F) = root and foliar application, AA = ascorbic acid. Si = silicon and SA = salicylic acid.

*Each number is means of three replications.

* Means followed by different letter(s) differ significantly according Duncan's multiple range tests, P ≤ 0.05.

(F) where recorded 19.77, 19.16, 19.03, 18.16, 16.42 and 14.28 respectively.

Results of Polyphenol oxidase activity (Unit. Min-1.gm-1 f w.) estimation as demonstrated in Figure 3 also there is reasonable increased with treatments and reached the pick in SA(R+F) 9.067 followed by AA(R+F) 8.6, SA(R) 8.167, SA(F) 7.6 then Si(R) 6.3, Si(R+F) 6 whereas foliar application of ascorbic acid and silicon were less effect and recorded 4.7 and 4.167.

Discussion

The salicylic acid (SA) application, especially roots and foliar represented better treatments for reducing the effect of nematode on chlorophyll. The SA played a role in defense as phytohormones have a role in photosynthesis (Hayat *et al.,* 2010), and these results agreed with our results in this trial. Results agreed with (López-Gómez *et al*., 2015), who state that Chlorophyll contents mostly decline with increasing nematode population density.

Chlorophyll content measures photosynthesis activity and nitrogen content in the plant. The decrease in chlorophyll due to root-knot nematode's influence interferes with water and nutrient transport through the infected plant (Melakeberhan *et al.,* 1987; Kirkpatrick *et al.,* 1991; Carneiro *et al.,* 2002). Thus better chlorophyll content in treatment indicates that treatment reduced the nematode effect on the cucumber plant.

Salicylic acid has a role in various enzyme activities and bio-productivity, which helps improve growth criteria besides decreasing the effect of infection (Hayat *et al.,* 2010).

The results of growth criteria corresponded with many researchers. They stated that the application of SA growth parameters could significantly enhance the dry shoot weight, root length, shoot weight and fresh root weight, and chlorophyll (Bakr *et al.,* 2018; Mukherjee *et al.,* 2012; Mostafanezhad *et al.,* 2014, and Moslemi *et al.,* 2016). New research performed by Ami and Shingaly (2020), found that SA application improves the growth criteria.

The effect of SA on infection reduction agrees with those recorded by Bakr and Hewedy (2018). Their study of systemic resistance induction in tomato against root-knot nematode by applied Salicylic acid (SA) at 50 mM and results demonstrated the highest reduction in number of galls, egg masses, females/tomato root system and number of second stage juveniles.

Reduction in infection criteria due to SA application attributed to its role as a signaling molecule involved in both reactions at infection sites and local defense and its role in the induction of systemic resistance. Although SA can serve as a long-distance messenger signaling in presence of any stress factors such as pathogen, so it's synthesis and accumulation are necessary requirements for defense responses (Walters *etal.,* 2013) as well as salicylic acid (SA) considers phytohormone which Salicylic acid (SA) is a key plant hormone required for establishing resistance to many pathogens is one of the most essential signal molecules have role in activator defense responses and/or inducing plant cells for response against pathogen infection (Ding & Ding, 2020). Researches also coincide with our results that silicon played important role in reducing the effect of biotic and abiotic stress in vegetable crops including root knot nematodes, researches illustrate that silicon lowers the effect of nematode on cucumber and many other vegetable crops by decreasing infection parameters. (Kaushik & Saini, 2019). Reduction in the nematode population was observed in the presence of Si under greenhouse conditions (Zhan, *et al.,* 2018). In the presence of silicates several studies have shown a reduction in the nematode population of the root-knot nematodes which is agreed with our results. Lower number of eggs, juveniles, and galls (Roldi *et al*., 2017). As well as when silicon is made available galling (root-knot) caused by *Meloidogyne* spp. are less severe which resulting in less disease severity and slower disease progress. Fortunato *et al.* (2015).

Effect of Silicon attributed to its deposition in plant cell walls acts as a physical barrier to avoid the penetration of plant pathogens (Debona *et al.,* 2017).

Many researchers result agreed with our results, applied Ascorbic acid in different way and their result also showed effect of AA on root-knot nematodes, application of ascorbic acid at 2000 ppm resulted in 100 % mortality of *M. javanica* juveniles after 7 days exposure and reduced the egg hatching Osman (1993).

In-vitro use of ascorbic acid 1250, 2500 and 5000 ppm increased the mortality of M. javanica juveniles up to 78.9 %. Infection criteria also decreased Abdel-Momen, *et al.* (2005) this results agreed with our result that AA effect on *M. javanica* result in a reduction in infection criteria.

Ascorbic acid also had practicable effect on decreasing infection criteria and increasing resistant, artificial increase in ascorbic acid concentration effected in transforms susceptible plants into more resistance against root-knot nematodes resistant ones (Farahat *et al.,* 2015. Ascorbic acid have role in synthesis of mitochondrial hydroxyproline proteins, which control the development of cyanide-resistant respiration. Arrigoni, *et al*. (1979).

This result also cooperates with researches stated that SA drastically increased activity and synthesis of defense enzymes peroxidase and Phenol oxidase in tomato plants Infected by root-knot nematodes (Bakr & Hewedy, 2018). Results agreed with other researches their results showed that Salicylic acid increased the level of polyphenol oxidase and phenolic compounds, phenylalanine ammonia lays, peroxidase, accumulation in the root system of tomato after inoculation with *M. javanica* Mostafanezhad, *et al*. (2014) as well as SA application increased some defense compounds such as hydrogen peroxide and peroxidase activities in root of cucumbers infected by *M. javanica.* Siahpoush, *et al.* (2011). Salicylic acid SA promote and increase activity of many enzymes such as cytoplasmic peroxidase phenylalanine ammonia lyase and catalase, which they have effect in defense response of plants against infection. Nikoo *et al.* (2014).

Raise the number of phenolic substances by treatments in means more defenses and that is obvious in reducing infection criteria that is because phenolic compounds have role in defense against pathogens and according to reviews involve in resistant against nematodes attack Ohri and Pannu (2010). Ascorbic acid also had acceptable effect in increasing defense enzymes and phenolic substances this might because of the artificial increase in ascorbic acid concentration effected in transforms susceptible plants into more resistance against root-knot nematodes resistant ones. Ascorbic acid have role in synthesis of mitochondrial hydroxyproline proteins, which control the development of cyanide-resistant respiration. Arrigoni *et al.* (1979.)

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