Potentials of commercial organic and biological fertilizers for inducing systemic resistance to the RKN Meloidogyne incognita in tomatoes

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Abstract

A study was conducted to test the possibility of inducing resistance in tomato plants using chemical agents (soil sterilizer), biological preparation, biocide and commercial preparation of seaweed extract. The root system was separated into two parts in two pots for each plant. After a week of plant stability, treatments were carried out separately, where young knot nematodes were injected into one part while the treatment was added to the other part of the root. The effect of each treatment in inducing plant resistance was evaluated after 6, 11 and 16 days of treatment depending on the concentration of resistance or pathogenic enzymes, CAT and POD, and the estimation of total phenolics and the extent of the effect of treatments on the percentage of chlorophyll in the plant. The results showed that the compound fertilizer preparation was the most efficient in increasing the concentration of POX and CAT, especially clearly after 11 and 16 days of treatment. Infected plants generally recorded higher levels of resistance enzymes than uninoculated plants even in the presence of treatments. Treatments with the presence of infection led to raising the levels of POD, CAT and total phenols to significantly higher values in most cases than those recorded in infected or untreated plants. The effect of treatments on the penetration and development of root-knot nematodes was observed, as higher penetration rates were recorded in the untreated inoculated control treatment, which then showed the development of juveniles to G3 and G4 after 16 days of inoculation, which was not recorded on the roots treated with the experimental factors. Abamectin showed relatively lower effectiveness than the rest of the treatments, while no knots were recorded in the Biohealth and Saviour-C soil sterilizer treatments. The study indicates the possibility of dipping tomato seedlings upon planting with any of the studied preparations to protect the plant from infection for at least the two weeks following transplantation of the seedlings.

Keywords: Biocontrol, defense enzymes, PPN, soil borne pathogens, Solanum

Introduction

Root-knot nematodes are among the most important pests of tomato cultivation, especially in tropical and subtropical regions and even arid regions with hot climates for most months of the year. This makes it difficult to use resistant varieties in these regions due to the breakdown of resistance resulting from exposure of the plant to high temperature [4, 8]. Hance, in most cases, if not always, the use of pesticides is the optimal solution to control root-knot nematodes to maintain stable production levels [6 .[better control than that provided by any pesticides after planting or in the middle of the season [30]. It has been found that early treatment of soil or seedlings with organic preparations or biofertilizers provides protection to the plant while improving growth and yield, and reduces nematode densities at the end of the season [6]. This includes preparations of biological origin such as Abamectin, which has been found effective in reducing infection indicators by various nematode species and on a broad scale against

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root-knot nematodes on different crops [10, 14, 15, 28]. Similarly, organic fertilizers and preparations containing Humic acid, including commercial algae extract, were found to reduce RKN populations with a significant improvement in growth and yield [7]. In the same context, preparations with more than one biological and organic factor with essential nutrients were always better than any individual factor in terms of reducing nematode infestation beside being more efficient in improving growth indicators and yield components in the treated crops [2, 9, 12, 19, 25]. One of these products is BioHealth, which consists of the bacteria Bacillus ceareus, the fungus Trichoderma sp., Seaweed extract, and a high percentage of humic acid with micronutrients. In general, all organic or biological preparations or their mixture do not show a specific mechanism against nematode infestation. Some are affecting nematodes mobility or nematode's ability to locate the host plant [16, 28]. while others might have toxic effect to nematodes [22]. However, many organic and biological formulas were reported to be plant resistance inducers [3, 27]. At the same time, they increase nutrients availability for the plant with the possibility of inducing systemic resistance (ISR) in the plant against potential infection [24, 25.[

As for biological preparations, different mechanisms may appear depending on the agent used, which generally work by mechanisms of competition, direct parasitism, or secretion of secondary materials that have antagonistic affect to nematodes [25]. In most experiments, inducing plant resistance against nematodes were performed directly as either soil application of foliar spray regardless the direct effect of the tested substance on the nematodes. In such cases, it cannot be determined whether the reduction in nematode infection is caused by direct exposure to the substance or due plant resistance induced by the factor itself. Hance, separating the part treated with the substance from the part inoculated with nematodes might be the best way to evaluate a particular factor in inducing plant resistance. Therefore, this study was conducted to evaluate the indirect application of some chemical, biological and organic products in inducing systemic resistance to RKN M. incognita infection in tomato young plants .

Materials and Methods

The possibility of inducing resistance in tomato seedlings was tested using different biological, chemical or organic fertilizers. Tomato seeds (Super Queen) variety, which is locally used in the desert area of Najaf Governorate, were planted in planting dishes, watered and maintained in wooden shade conditions. After 35 days of germination, the seedlings were transferred to small cork pots, where each plant was planted in two pots by dividing the root system to be in the shape of an inverted Y letter (ʎ). After a week of plant stability, the treatments were carried out, as one pot of each plant was subjected to the treatment while the other pot was inoculated with 500 freshly hatched J2 of the RKN M. incognita. The treatments were Abamectin pesticide, Biohealth commercial fertilizer, Seaweeds extract SWE, or the commercial soil sterilizer (SAVIOR-C), in addition to same treatments in the absence of the nematode inoculum for each of the experimental factors and the positive control treatment inoculated with nematodes only and the negative control untreated-uninoculated, with 9 replicates to be taken at three periods of 6, 11, 16 days post treatment with a total number of 90 experimental units. The experimental

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measures (data) were collected at 6-, 11- and 16-days post treatment (DPT) in order to evaluate the effect of the treatments in inducing resistance based on the plant's content of resistance or pathogenicity related enzymes, Peroxidase (POD) and Catalase (CAT), in addition to the plant's content of total phenols, and chlorophyll A and B. The enzymatic activity of POD (Peroxidase) enzyme was estimated according to the method described by Marchetti et al. (1995) [20]. as the enzyme's activity was estimated using Spectrophotometer where the absorbance was measured at a wavelength of 436 nm, and the change in absorbance was monitored every 30 seconds for five minutes. Estimation of Catalase (CAT) enzyme activity was performed [1]. as the final mixture solution absorbs light in the Spectrophotometer at a wavelength of 240 nm, noting a decrease in absorbance over time. Plant content of total phenols (%) was also estimated in the dry samples by the method of 80% methanol and 1% HCL to read the absorbance using the Spectrophotometer UV-Visible at a wavelength of 725 nm [32]. The leaf content of chlorophyll A and B in the plant sab (mg/100g-1 fresh weight) were also measured. The optical absorbance of the filtrate was measured at wavelengths of 645 and 663 nm by the aid of UV-Visible Spectrophotometer [29 .[

Effect of the treatments on RKN penetration and development

Plants were taken after 6, 11 and 16 days, as infected roots were gently washed and subjected to staining [5]. By the aid of a dissecting microscope at 2X magnification, the stained nematodes in the root tissue were observed and the nematodes penetration and development were determined and compared among treatments.

Statistical Analysis

Genstat 12th (VSN, 2009) [33] statistical analysis tool was used for data analysis and performing analysis of variance ANOVA table. Means were compared among treatments according to Duncan's multiple range tests ($P \leq 0.05$) wherever appropriate.

Results and discussion

The results showed that tomato plant content of antioxidant enzymes Proxidase POD, Catalase CTA and total phenols differed among the experimental factors under study (Table1). Findings indicated that the activity levels of peroxidase (APD) and catalase (CAT) enzymes in healthy plants recorded low levels after 6, 11 and 16 days, with a significant decrease in most cases compared to the untreated inoculated with RKN M. incognita J2s, which recorded higher rates for both enzymes after the same periods. The two enzymes increased significantly in all the infected treatments compared to the uninfected same treatments. The pathogenic enzyme POD recorded its highest value after six days in the RKN inoculated tomato plants treated with Biohealth biofertilizer, followed at a similar level by SWE treatment, then the soil sterilizer SAVIOUR-C and Abamectin, respectively. While the lowest rate POD activity was always recorded in the healthy uninoculated control in all the periods post treatments (Table 1). The levels of CTA enzyme increased with the effect of infection with a significant difference from the uninfected plant (healthy) in general. The highest level of CTA enzyme activity (152.25) was recorded in the bio treatment inoculated with nematodes after 6 days of treatment with a significant difference also from healthy plants of the same treatment .

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Table2. Effect of organic or biofertilization and chemical treatments in induction of resistance enzymes peroxidase and catalase and total phenols in tomato plants inoculated with the root-knot nematode Meloidogyne incognita

	POD			**CAT**			**T. phenols (%)**		
Treatments	6 DPT	11 DPT	16 DPT	6 DPT	11 DPT	16 DPT	6 DPT	11 DPT	16 DPT
Healthy	24.90± 1.12 f	37.36±1.52 g	29.88± 1.14 g	103.13±1.22 g	24.71±1.10 d	15.50±1.12 e	0.240± 1.33 c	0.157±1.09 e	0.674±2.55 d
Infected	31.33± 2.02 de	47.01±1.09 ef	37.60± 2.22 ef	116.2 ±2.23 f	32.69±1.35 b	21.81±1.12 d	0.312± 2.08 b	0.324±1.11 c	0.769±1.89 c
Ab/N	38.84± 1.05 b	58.26±2.17 b	46.60± 1.13 b	147.72±1.22 b	34.84±1.16 b	26.7±1.12 c	0.263± 1.11 c	0.132±2.29 e	0.352±1.34 g
Ab	31.06± 0.38 de	46.60±1.61 ef	37.27± 1.26 ef	130.57±2.63 ef	25.83±2.21 d	15.81±1.12 e	0.238± 0.33 c	0.125±1.13 e	0.437±1.05 f
BioH/N	44.86± 2.11 a	67.3 ±1.12 a	53.84± 1.34 a	152.25±1.08 a	46.78±2.32 a	36.64±1.12 a	0.247± 1.06 c	0.31±0.40 c	0.332±1.14 g
BioH	36.25± 1.31 bc	54.38±2.14 cd	43.51± 2.18 cd	144.57±1.33 bcd	33.13±1.33 b	20.5±1.12 d	0.233± 1.14 c	0.240±1.27 d	0.267±1.09 h
SWE/N	33.44± 1.09 cd	50.17±1.12 de	40.13± 1.24 de	146.93±2.22 bc	44.83±2.68 a	38.49±1.12 a	0.381± 1.20 b	0.549±1.10 a	0.892±1.12 a
SWE	29.87± 2.61 de	44.81±1.08 f	35.84± 1.36 ef	138.80±0/63 cd	36.15±1.09 b	31.88±1.12 b	0.341± 2.25 b	0.485±2.70 b	0.848±2.27 b
SAV/N	38.16± 1.22 b	57.25±1.14 bc	45.8±1.52 bc	133.57±1.05 de	42.87±1.28 a	38.50±1.12 a	0.482± 1.08 a	0.358±1.22 c	0.496±1.15 e
SAV	30.25± 2.23 de	45.38±1.03 ef	36.3±2.18 ef	128.81±2.04 ef	31.48±2.07 bc	26.16±1.19 c	0.448± 1.12 a	0.328±1.06 c	0.634±1.09 d

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LSD ($P\leq 0.05$)	3.392	4.873	4.226	12.323	4.315	4.225	0.047	0.042	0.053

*Values are means of three replications. Means within a column followed by different letter(s) are significantly different according Duncan's multiple range test ($P\leq 0.05$). Treatments are: uninfected (healthy), infected by root-knot nematodes (N), treated with Abamectin (Ab), Biohealth (Bio), Seaweed extract (SWE), or soil sanitizer SAVIOUR-C

This was continuing in the similar manner regardless of the period after treatment, followed by the Abamectin and SWE treatments, then the soil sterilizer SAVIOR-C treatment, all of inoculated treated recorded higher values for CTA enzyme activity than the positive control (inoculated untreated). Similar results for all the treatments recorded after 11- and 16- days post treatment, where in all cases it was noted that the enzyme activity decreased with the increase in the period after treatment, as it decreased by 75-85% in the period 11 and 16 days after treatment in all treatments regardless of the presence or absence of infection. The results of the same table (1) also indicate that the treatments differed in the plant content of total phenols, as all treatments recorded high levels compared to healthy plants, although some treatments, especially ab and bio treatments, did not differ from the uninfected control, while the same treatments recorded a significantly lower phenol percentage in the leaves than the infected control treatment, as the latter did not differ from the algae extract treatments, while the pesticide treatment recorded the highest values of phenol and a significant difference from all treatments after 6 days of treatment. However, after 11 days of treatment, the SWE treatment recorded the highest value of phenol, which continued even after 16 days with a significant difference from all treatments. It was also found that the uninfected SWE treatment also led to higher levels of leaf phenol compared to the other treatments. In general, it was noted that the level of phenol in one treatment increased in the presence of nematodes than in their absence, except for the Abamectin treatment after 11 days, which recorded almost equal values regardless of the presence of infection.

It was found (Table 2) that the plant content of chlorophyll A or B differed among treatments and also differed according to the period after treatment, as the highest value of chlorophyll A in the early stage was recorded in the pesticide treatment and the control treatment for healthy plants, with slight differences in most cases from the other treatments, while most treatments did not differ in the value of chlorophyll A after 11 days of treatment compared to that recorded after 16 days. The highest chlorophyll A value was of 25- to 26 mg 100g-1FW in the Biohealth treatment, with a significant difference from all other treatments that recorded values ranging from 12.88 in the infected control treatment to 22 mg 100g-1FW in the chemical SAVIOUR-C treatment. While, no clear differences were recorded among treatments in case of chlorophyll B, as lower values were recorded in most infected treatments than in the uninoculated treatments. It was also noted that the chlorophyll B index increased at least twice after 11 days of treatment compared to 6 days, then returned to decrease by approximately 50-80% after 16 days of treatment, in which Biohealth recorded significantly the highest value compared to the rest of the treatments. It was also noted that

Chlorophyll B decreased significantly to the lowest value in the healthy treatment .

As for the effect of treatments on RKN development and galling symptoms, M. incognita in many cases was not determined even there were many developing small gall formation in the treated tomato roots at 16 DPT. while M. javanica was more developed reaching the swelling 'sausage' stage in the control untreated infected tomato root (Fig. 1.8 B). Tomato infected treated roots at late stage 16 DPT showed irritated root tissue stained randomly but no galls were detected especially in the treatments of soil sanitizer SAVIOUR-C and biological fertilizer Biohealth (Figure1.(

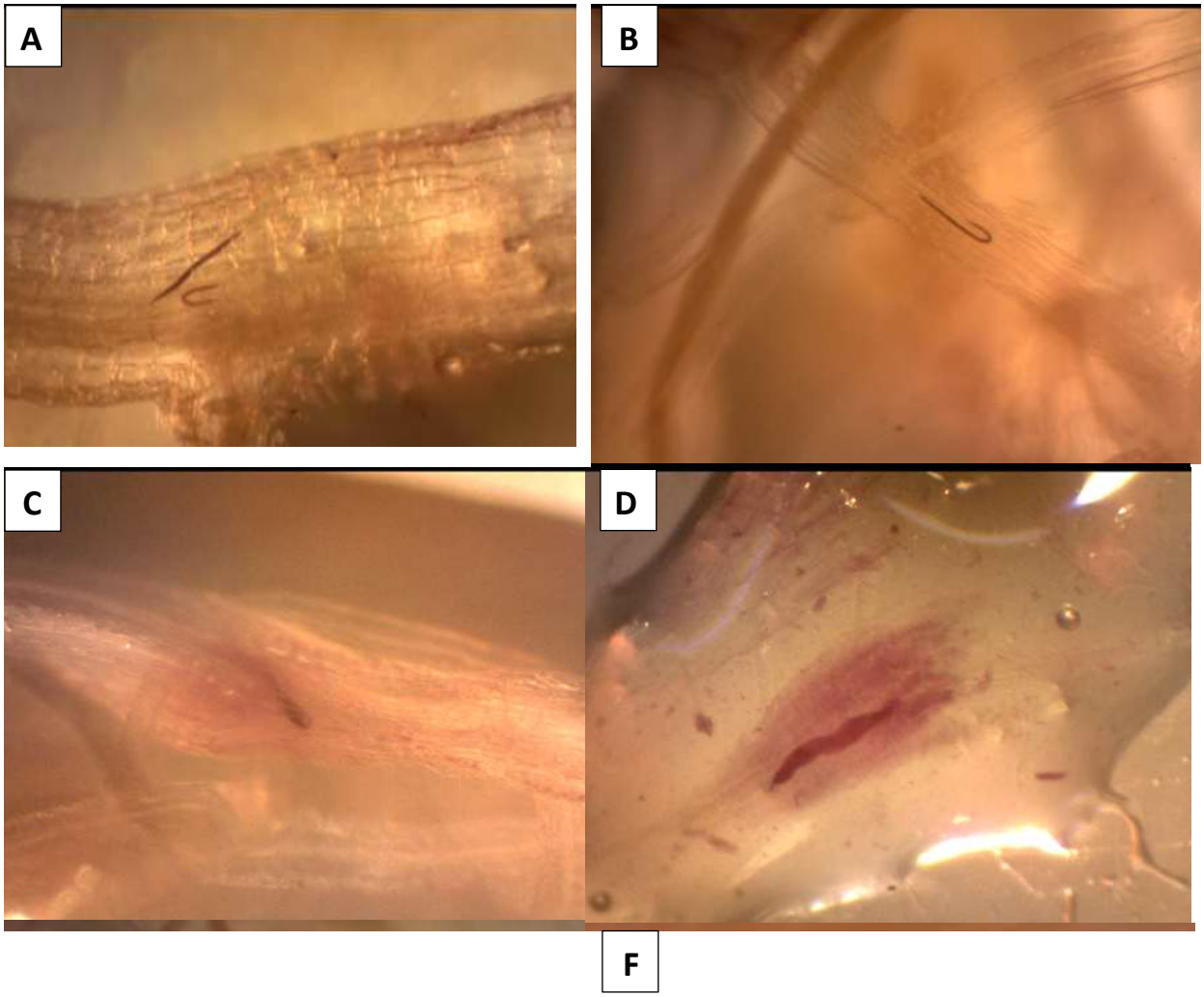
Table2. Effect of organic or biofertilization and chemical treatments on shoot content of chlorophyll A and B in tomato plants inoculated with the root-knot nematode Meloidogyne incognita after three periods (days) post treatment (DPT)

	Chlorophyll A			**Chlorophyll B**		
Treatments	6 DPT	11 DPT	16 DPT	6 DPT	11 DPT	16 DPT
Healthy	11.9 a	25.81 a	15.57 c	13.91 ab	41.38 c	8.73 e
Infected	10.74 ab	24.11 ab	12.88 d	17.18 a	39.16 c	12.51 d
Ab/N	9.50 ab	26.42 a	21.10 b	15.00 a	44.87 ab	23.34 b
Ab	11.08 ab	25.41 a	21.86 b	13.26 ab	47.01 a	23.30 b
BioH/N	9.88 ab	26.66 a	25.00 a	10.35 bc	48.41 a	29.32 a
BioH	8.06 bc	25.95 a	26.38 a	14.20 ab	48.74 a	30.94 a
SWE/N	7.02 bcd	23.99 ab	20.04 b	7.54 cd	40.96 c	13.31 d
SWE	6.58 bcd	24.73 a	16.09 c	8.11 cd	36.59 cd	14.06 d
SAV/N	10.18 ab	24.99 a	22.00 b	11.30 bc	47.7 a	23.09 b
SAV	13.53 a	26.32 a	20.82 b	13.73 ab	38.36 cd	18.58 c
LSD ($P \leq 0.05$)	1.712	2.022	2.041	1.862	2.662	1.821

*Values are means of three replications. Means within a column followed by different letter(s) are significantly different according Duncan's multiple range test ($P \leq 0.05$). Treatments are: uninfected (healthy), infected by root-knot nematodes (N), treated with Abamectin (Ab), Biohealth (Bio), Seaweed extract (SWE), or soil sanitizer SAVIOUR-C

It is known that plant infection with knot nematodes often, if not always, causes slight or imperceptible damage between plant cells, and despite that, there is a possibility of identifying the infection early after the nematode invasion and penetration of the roots. Especially, by relying on the high oxidation activities in the cells as a result of the response to the infection, which is reflected by an increase in the content of the infected tissue of the pathogenic or resistance enzyme POD (H2O2), which is often considered one of the enzymes that induce

high resistance, especially in the resistant plant, which is followed by the induction of one of the resistance mechanisms, including cell death. Since the penetration of the nematode into the root does not generally differ in the resistant plant from that in the sensitive plant, but what follows the penetration in the nematode's attempt to form feeding sites, the plant's response differs, as it is noted that the level of the enzyme (POD) rises in the resistant plant several times what is found in the sensitive plant at the same stage [26]. It is also noted that the level of the enzyme, regardless of the extent of the plant's resistance, rises highly in the infected plant as a result of the infection compared to very lower levels in the uninfected (healthy) plant [26].



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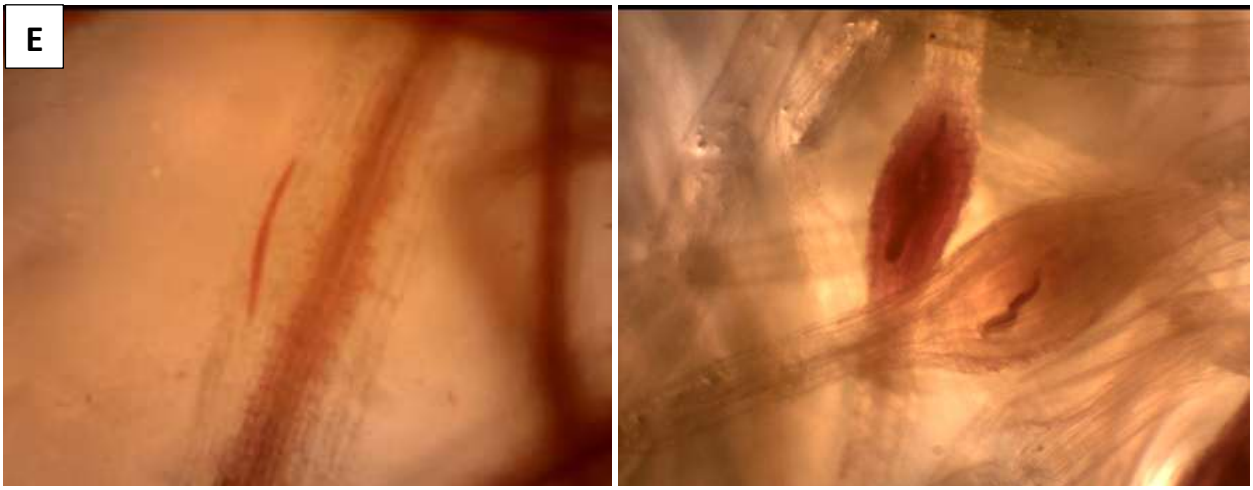

Figure1. Various developmental stages of M. incognita on tomato young plants at 6 DPI days post inoculation (A and B), 11DPI (C and D), and 16DPI (E and F). M. incognita migrating or swelling in the cortex of control untreated plant roots (B and D) more developed swelling after 16 days post inoculation (F). They were barely found and developed due to treatments (A, C and E.(

Studies indicate high concentrations of hydrogen peroxide (POD) in infected roots in the early stages of infection, which generally range between (3-20 days) after infection [24]. High levels of (POD) give clear evidence of the plant resistance activities expressed by the plant through the subsequent local death of infected cells in addition to stimulating defenses in neighboring cells [21]. This generally refers to responses and interactions of plant tissues against the pathogen indicated by the presence and increase of POD and CTA enzyme, which do not appear or may appear slightly in susceptible plants that often record lower activities [11, 24]. This indicates that adding any biological or chemical component to the plant, root or vegetative, which leads to raising the level of the enzyme (POD) in the plant content means ensuring that this substance has led to the induction of resistance towards this component or towards other factors, which indicates the possibility of inducing resistance with a specific substance. As induction factors may differ according to their compatibility or the extent of plant response towards certain reactions after absorption from plant cells. This is consistent with the results of the study that showed the possibility of inducing resistance of tomato seedlings against RKN Meloidogyne incognita, as the level of activity of POD and CTA, and in most cases of total phenols varied according to the treatments that recorded their highest levels in the Biohealth biofertilizer preparation, as it is a compound preparation containing more than one biological substance from the bacteria Bacillus subtilis and the bio-fungus Trichoderma harzianum in addition to the preparation's content of humic acid, seaweed extract and mineral nutrients [18, 26.[

Lower levels of enzyme activity were also observed in plant treatments with single agents such as Abamectin, Sea Weeds extract, or even chemical treatment with SAVIOR-C, a general soil and root disinfectant. This is consistent with the results of studies indicating that POD enzyme levels in induced plants

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increase in treatments consisting of more than one factor or also increase with the interaction of treatments for different factors [13, 17, 31.[

Conclusion

The findings showed that compound fertilizer Biohealth was the most effective in increasing plant defense enzymes POX and CAT significantly, especially after 11 and 16 days of treatment. Infected plants generally showed higher levels of defense enzymes than healthy plants regardless of the type of treatment. A clear effect of the treatments was observed on RKN M. incognita ability to penetrate and develop, noting that the highest penetration rates were in the untreated inoculated control treatment. Abamectin showed relatively lower effectiveness among the treatments, while root galling was not recorded in the Biohealth and Saviour-C treatments. The study concluded that pre-planting dipping tomato seedlings in any of the studied factors can protect the plant from infection for at least two weeks after planting.

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