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INTEGRATED MANAGEMENT OF *Meloidogyne incognita* ON TOMATO USING COMBINATIONS OF COMMERCIAL ABAMECTIN AND PLANT ACTIVATOR

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Abstract: One of the main pests of tomatoes is root knot nematodes and causes significant yield losses. The abamectin is a bio-based pesticide and plant activators is used stimulating systemic acquired resistance mechanisms. Determining their suppressive effects on nematodes and understanding their interactions may be important for better use in integrated management. The effect of abamectin (*Streptomyces avermitilis*, Abamax®) and plant activators (harpin and *Lactobacillus acidiophilus*) singly or in combination was tested against *Meloidogyne incognita* on tomato under controlled conditions. The experiment was established 5 days after transplanting of 35 days of tomatoes. ProAct Plus® (Harpin, 0.15 g/l), ISR-2000® (*L. acidophilus*, 1 ml/l) and Crop-Set® (*L. acidophilus*, 0.6 ml/l) were applied to the leaves by spraying, while Abamax® was applied to the soil. Nematode inoculation (1000 second juvenile larvae (J2)) was planned 72 hours after the first application of activators. The activators were applied to tomatoes 2 more times with 14 days intervals. After sixty days, plant height and fresh weight, root height and fresh weight, number of galls and egg masses, gall index, J2 soil density and lignification of leaves, stem and roots were evaluated. While the gall index was 4/0-5 index in plants treated only with nematodes, it was found to be 1.2/0-5 index in Abamax®. While 1.6 was found in Proact Plus®, 2.0 was detected in ISR2000® and Cropset®. No galls or egg masses were found in ProAct Plus®±Abamax®, ISR-2000®±Abamax® and Crop-Set®±Abamax®. The positive effect of abamectin alone on plant development was found to be higher than plant activators. Root fresh weight increased significantly in abamectin and plant activator combinations. Plant activators caused an increase in lignification and the highest level was found in Proact Plus®. Lignification was higher in combinations with abamectin. The highest lignification was in Abamax®±Proact Plus®. Combinations of harpin and *L. acidophilus* activators with abamectin may be a potential antagonism strategy against root-knot nematodes.

Keywords: Abamectin, *Meloidogyne incognita*, Plant activators, Harpin, *Lactobacillus acidiophilus*

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1. Introduction

Root-knot nematodes belong to the genus *Meloidogyne*, which has more than 98 species (Jones et al., 2013). Among them, *Meloidogyne incognita, Meloidogyne javanica, Meloidogyne arenaria* and *Meloidogyne hapla* represent 95% of the populations of all root-knot nematode species (Dong et al., 2012). They sensitize the plants to other pathogens and stress factors (Kyndt et al., 2013). Due to their fixed endoparasitic, wide host range, high reproductive rate and short life cycle, control of root knot nematode is more difficult than other plant pathogens (Quentin et al., 2013). The use of fumigants and nematicides is common in root knot nematode control. However, these pose a serious risk to both the environment and human health due to misuse, overuse and long persistence in soil and groundwater, which can adversely affect all food chains (Ntalli et al., 2011, Azlay et al., 2022). In recent years, studies have focused on environmentally friendly alternative controls that can help control root-knot nematode without harming non-target organisms (Degenkolb and Vilcinskas, 2016). Biological control is the most effective alternative found to date for the control of root-knot nematode and has potential for integrated pest management (Kumar and Arthurs, 2021). In integrated pest and disease control methods, there has been an increased interest induced resistance in biological control (Saravanakumar et al., 2007). The induced resistance is the stimulation of the plant's defense mechanisms by a biotic or abiotic factors and induced resistance can be expressed as the activation of passive resistance mechanisms in the plant, not the creation of a non-existent resistance (Van Loon et al., 1998). Resistance is realized by recognizing and activating the elicitor molecules of the pathogen and promoting oxidative reaction, lignification, hypersensitive response, PR proteins and any of the changes in plant metabolism (Chaube and Pundhir, 2005, Barutçu, 2006). Plant activators are defined as "substances that activate the natural defense systems of

plants, enable them to make better use of nutrients, help to protect them from stress conditions and similar external factors and factors, and/or have natural and/or chemical strengthening, resistance-enhancing, soil structure-regulating properties that positively affect yield and product quality and carry one or more of these properties together". Plant activators enable plants to show resistance against diseases by stimulating Systemic Acquired Resistance (SAR) mechanisms (Durrant and Dong, 2004).

Harpins are naturally occurring proteins from a novel group of compounds first reported from *Erwinia amylovora* (Wei et al., 1992). Harpin proteins, including harpinEα and harpinαβ, elicit the expression of genes involved in the hypersensitive response, enhancement of plant growth and activate an induced systemic defense response (Wei and Beer, 1996). This response has been associated with increased resistance to pathogens and some other pests in plants. As a result of these discoveries, commercial plant activator products containing harpin proteins such as Messenger®, N-HIBITTM®, Mighty PlantTM® and ProActTM® have been developed (Kirkpatrick et al., 2006). In Türkiye, the bioactivator whose active ingredient is harpin was previously available in the market as Messenger®, but is now sold as ProAct® (Şener, 2015). Infection with *M. incognita* was induced in cotton cultivars in which transgenically expressed harpinEa constructs (PE58) were created, compared to the susceptible cultivar. In each experiment, selected transgenic cultivars produced on average 56% to 62% fewer eggs and 72% to 81% fewer J2 compared to the susceptible control cultivar Coker (French et al., 2006). Kirkpatrick et al. (2006) applied 2 harpin proteins (EBC-151ST and EBC-152) in cotton by seed spraying and 1 by foliar spraying (EBC-351A), resulting in less root rot and fewer *M. incognita* eggs and J2 on average compared to control plants. In multi-location field trials conducted in Minnesota (MN), a sensitive cultivar (Pioneer 91M70) treated with N-Hibit HX-209® was found to have 4% higher yields than untreated plants (Lisa et al., 2007). In contrast, field trials in Iowa using seeds coated with N-Hibit HX-209® found no effect on yield and soybean cyst nematode (SCN) egg population at the end of the season (Tylka and Marett, 2008).

Lactic acid bacteria (LAB) also have antagonistic activity against pathogenic bacteria and fungi, making them ideal for developing biocontrol agents for use in plants (Trias et al., 2008; Jang et al., 2011; Guarner et al., 2012; Lim et al., 2018). Compounds such as organic acids, hydrogen peroxide, bacteriocins and lipid and amino acid metabolites produced by LAB are among the antimicrobial factors (Kormin et al., 2001). Nowadays, LAB attract great attention in the agricultural industry as an alternative to problems such as antibiotic resistance and pesticide residues. It is known that *Lactobacillus* species act as an antagonistic agent and exhibit antimicrobial activity (Hamed et al., 2011). However, there are not many studies on the nematicidal activity of *Lactobacillus* spp. on phytopathogenic nematodes. Seo et al. (2019) found that *L. brevis* was effective on root-knot nematodes. Crop-Set® is Türkiye's first licensed activator and the first natural plant activator with an organic license. It contains *L. acidiophilus* liquid fermentation product, plant extract, manganese sulfate, iron sulfate and copper sulfate. Crop-Set® increases the plant's ability to utilize nutrients in an environmentally safe way, thereby optimizing fruit and vegetable yields and improving quality and uniformity. With its bacteriocins and organic acids, it stimulates the plant immune system and increases plant resistance in the fight against harmful microorganisms (Şener, 2015). ISR-2000 is a fermentation product of *L. acidophilus* and contains yucca plant extract, yeast extract, riboflavin, benzoic acid, nicotinamide and thiamine. ISR-2000® increases the activity of enzymes such as chitinase, gluconase and peroxidase in the plant. After the stimulation occurs, the plant remains at the highest level of alert against a possible subsequent attack and thus can best defend itself against pathogenic invasion (Tosun and Ergün, 2002, Koca, 2003). *Lactobacillus acidophilus* is a gram (±), rod-shaped, non-spore forming lactic acid bacteria (Suraporn et al., 2015; Urmann et al., 2016). It was found that the nematode *Caenorhabditis elegans* when fed with *L. acidophilus*, *Enterococcus faecali*s and *Staphylococcus aureus* infections prevented (Kim and Mylonakis, 2012).

Abamectin is one of the alternative biocathional mediators belonging to the avermectin group of macrocyclic lactone metabolites produced by natural fermentation of *Streptomyces avermitlis* bacteria. Abamectin is used as insecticides, acaricides and nematicides in vegetables, fruits and field crops (Khalil, 2013). The mode of action of avermectins is to block the transmission of electrical activity in nerves and muscle cells by stimulating the release and binding of gamma-amino butyric acid (GABA) at nerve endings (Roder and Stair, 1998). This causes an influx of chloride ions into the cells (activating or opening the glutamate-gated chloride channel), which leads to hyperpolarization and subsequent paralysis of neuromuscular systems (Cully et al., 1994; Burkhart, 2000) and subsequent death. In nematodes, GABA receptors are found in neuromuscular and central ventral ganglia. GABA has also been reported in second juvenile (J2) larvae of *Globodera rostochiensis* and *M. incognita* (Stewart et al., 1994). Seed, soil and foliar applications of abamectin were found to have suppressive effects on nematodes. Seed treatment with abamectin reduced J2 penetration into roots, resulting in lower colonization and reproduction of *M. incognita* in cotton and cucumber plants (Becker et al., 2006; Bessi et al., 2010). Abamectin (Vertemic 1.8% EC) has proven its nematicidal activity as a soil application suppressing root-knot nematodes on different vegetable crops (Hamida et al., 2006; Khalil, 2012; Saad et al., 2012). In addition, it was effective against *Ditylenchus dipsaci* in

garlic, reducing nematodes per cm^2 tissue (Becker, 1999).

In this study, the effects of single abamectin and plant activators (containing harpin and *L. acidophilus*) application and combinations with each other on the infection of root-knot nematode, *M. incognita* in tomato, plant growth parameters and plant ligninization were investigated under controlled conditions.

2. Materials and Methods

2.1. Materials

Harpin 3% WG (ProAct Plus®, AMC-TR) and *L. acidiophilus* fermented content ISR-2000® (Alltech Crop Science) and Crop-Set® (Alltech Crop Science) plant activators and abamectin (Abamax 50 SC®, Rotam) were used in the study. Plant activators and abamectin were purchased commercially. The study will be conducted with 35 days old Özkan F1 tomato seedlings which susceptible root knot nematode. The ISP root knot nematode isolate, which was pure cultured and morphologically identified in previous studies (Göze et al., 2022).

2.2. Mass production of root-knot nematode

Mass production was carried out with 15 plants under controlled conditions (24±1 °C, 60%±5% humidity). The J2s were obtained from the pure cultivated tomato roots by removing the egg masses under a stereo binocular microscope using the petri method. Previously, 1000 J2s were inoculated in 1000 microliters of water by making small holes in the soil near the root collar of each of the Tueza F1 tomato seedlings that were transplanted in pots. Plastic pots with a volume of 250 ml and sterile soil mixture containing 68% sand, 21% silt and 11% clay were used. Mass production was terminated 8 weeks after inoculation.

2.3. Preparation of nematode inoculum

After washing the roots of Tueza F1 tomato variety in tap water, egg masses were taken from the roots under stereo microscope and incubated in a petri dish in water at 25±2°C for three days. After three days, J2s emerging from the eggs were counted under a light microscope (Göze Özdemir et al., 2022) and 1000 J2s placed in 1 ml tubes (Özdemir and Gözel, 2017).

2.4. Pot experiments

This study, which was carried out to induce resistance in tomato plants by using plant activators alone and in combination with abamectin and to investigate the possibility of using it in the control of root-knot nematodes, consisted of 9 applications (Table 1). The study was established in a randomized plots experimental design with 5 replications for each application. Each replicate was planted with 1 Özkan F1 tomato seedling. The seedlings were transplanted into 250 ml plastic pots containing 300 g of sterile soil mixture (68% sand, 21% silt and 11% clay). The experiment was established 5 days after transplanting. ProAct Plus®, ISR-2000® and Crop-Set® were applied to the leaves by spraying, while Abamax was applied to the soil. The dosage of plant activators was prepared by using the label information. The recommended dose was set at 0.15 g/l for ProAct Plus®, 1 ml/l for ISR-2000® and 0.6 ml/l for Crop-Set®. The activators used in the application were applied to tomato plants 2 more times with 14 days intervals after the first application date of the activators as specified in the label information (Şener, 2015). The application was carried out 3 times in total until the experiment ended. Nematode inoculation was planned 72 hours after the first application of activators as a spray on plant leaves. A thousand of *M. incognita* J2 with 1 ml of water were inoculated into holes drilled near the root of tomato in pot. Plants with only nematode inoculation and plants without nematode inoculation were used as controls. Additionally, no activator was applied to plants without nematode inoculation.

Table 1. The applications of experiment

1	ProAct Plus® alone
2	ISR-2000® alone
3	Crop-Set® alone
4	Abamax® alone
5	ProAct Plus®± Abamax®
6	ISR-2000®±Abamax®
7	Crop-Set®± Abamax®
8	Nematode (+) Control
9	Nematode Free (-) Control

The experiment was terminated 60 days after nematode inoculation. Afterwards, the plants height and fresh weight measured, roots remove the soil and washed with clean water. Then, root height and root fresh weight values were taken. Later, the number of gall and egg masses in the roots were counted under stereo microscope. Evaluation was done 0-5 scale (0= no gall, 1= 1-2 gall, 2= 3-10 gall, 3= 11-30 gall, 4=31-100 gall, 5= more than 100 gall).

SPSS (version 20.0) program was used for statistical analysis of the data obtained as a result of the experiment, and analysis of variance (ANOVA) was performed to test the differences between the means. Means were compared by Tukey HSD test at $P \leq 0.05$.

2.5. Determination of Lignin Synthesis

Lignin accumulation in leaves, roots and stem parts of tomato plants, in which resistance was promoted by the application of plant activators, was determined by phloroglucinol/hydrochloric acid (HCl) test (Şener, 2015).

In pot experiments, the treated tomato plants were removed from the pots without damaging the root zone and washed with water. Certain tissues were taken from the roots, stems and leaves of these plants. The chlorophylls in the infected tissues were removed in 100% methanol containing 1% phloroglucinol at room temperature (20^oC) for 1 night. The whitened tissue samples were kept in chloral hydrate (2.5 g/ml) for at least 24 hours to make the tissues transparent.

Chlorophyll was removed with the help of methanol and the tissues cleaned with chloral hydrate were placed on a sterile slide and 1-2 drops of concentrated HCl solution was added and kept for 10 minutes. At the end of the waiting period, a few drops of 50% glycerol solution were added on the tissues and the coverslip was closed. The covered coverslips were examined under a light microscope. Since the stained tissues lost their color within 3-5 hours, the samples were examined under a light microscope immediately after staining. It was observed that 10 minutes after HCl was added to the infected tissues, the lignified structures turned a dark pink color (Şener, 2015).

3. Results

The number of galls, number of egg masses, gall index and J2 density in the soil of control plants treated with nematodes only were significantly higher than all treatments (P≤0.05). The number of galls and egg masses in the roots of Abamax® treated plants were lower than Cropset®, ISR2000® and Proact® application. When the plant activators were compared among themselves, the lowest number of gall and egg masses was found in Proact® application. The number of gall and egg masses in ISR2000® application was higher than Cropset® application. In the combinations of plant activators with Abamax®, gall and egg masses were not detected in the roots. While the highest J2 density in soil was determined in the nematode control, J2 density decreased significantly in single and combination treatments (P≤0.05). However, there was no significant difference (P≤0.05) between applications in terms of J2 density in soil (Table 2).

There was no statistically significant difference in gall index between Cropset®, ISR2000®, Proact® and Abamax® application (P≤0.05). While gall index was lower in combination application than single application, there was no statistically significant difference in gall index between ISR2000® ± Abamax®, Cropset® ± Abamax®, and Proact® ± Abamax®combination applications (P≤0.05). Although the suppressive effects of plant activators alone on the nematode were determined, the control effect was significantly increased in combination with Abamax® (Table 2).

The lowest plant height was found only in the nematode treated control plants. When compared to the plants without nematode inoculation, plant height decreased significantly. The highest plant height was found in Abamax® application. Plant height was lower in Cropset®, ISR2000® and Proact® applications than Abamax application. In addition, there was no statistical difference (P≤0.05) in plant height when the combination applications with Abamax® were compared with single. Compared to the nematode control, single applications of Cropset®, ISR2000® and Proact® had positive effects on plant height. However, the effect of Abamax® application alone was found to be high on plant height, while plant height was found to be lower in combination applications (Table 3).

The lowest plant fresh weight was found only in nematode treated plants. The highest plant fresh weight was found in Proact® application. Plant fresh weight of ISR2000® application was lower than Proact® and Cropset® applications. Abamax® combination of ISR2000® treatment had a positive effect on plant fresh weight. There was no statistically significant difference in plant fresh weight between the combinations of Cropset® and Proact® with Abamax® and single applications (Table 3).

When the plant activators were evaluated among themselves in the root length parameter, there was no significant difference between them (P≤0.05). Root length values of plant activators applied alone were found to be similar to Abamax® application. Although there was a numerical increase in root length values in combinations of plant activators with Abamax®, there was no statistically significant difference between them (P≤0.05). However, when compared with the nematode control, Abamax®, Cropset® ± Abamax® and Proact® ± Abamax® treatments had a positive effect on root length (Table 3).

Compared to the nematode control, root fresh weight was higher in Abamax® and combination treatments. Root fresh weight was higher in Cropset® treatment than ISR2000® and Proact® treatment. Plant activators had a positive effect on root fresh weight when applied together with Abamax®. The effect of Abamax® on root fresh weight was higher than that of plant activators applied alone (Table 3).

Table 2. Effect of applications on the development of *Meloidogyne incognita* on tomato root (Mean±Std. Error)

Application	Number of galls/root	Number of Egg masses/root	Soil J2 Density (100 g soil)	Gal Index(0-5)
Cropset®	4.6±0.7^{ab}*	4.3±0.5^{c}	18.0±5.8^{b}	2.0±0.0^{b}
ISR2000®	7.2±0.8^{b}	5.2±0.6^{b}	12.0±7.3^{b}	2.0±0.0^{b}
Proact®	1.2±0.3^{bc}	0.2±0.2^{e}	4.0±2.4^{b}	1.6±0.4^{b}
Abamax®	0.6±0.2^{bc}	0.6±0.2^{d}	2.0±2.0^{b}	1.2±0.4^{b}
Cropset®±Abamax®	0.0±0.0^{c}	0.0±0.0^{e}	0.0±0.0^{b}	0.0±0.0^{c}
ISR2000®±Abamax®	0.0±0.0^{c}	0.2±0.2^{e}	0.0±0.0^{b}	0.0±0.0^{c}
Proact®±Abamax®	0.0±0.0^{c}	0.0±0.0^{e}	2.0±2.0^{b}	0.0±0.0^{c}
Nematode (+) Control	47.2±2.4	42.8±2.7^{a}	55.6±60.7^{a}	4.0±0.0^{a}
Nematode-free (-) Control	0.0±0.0^{c}	0.0±0.0^{c}	0.0±0.0^{b}	0.0^{c}

*The lowercase letters in the same column indicate statistically differences between applications (P≤0.05).

Table 3. Effect of Applications on Tomato Plant Growth Parameters (Mean±Std. Error)

Application	Plant Height	Plant Fresh Weight	Root Height	Root Fresh Weight
Cropset®	33.9±1.29^{b*}	12.1±1.2^{ab}	17.7±0.7^{ab}	3.5±0.1^{abc}
ISR-2000®	34.3±1.3^{b}	8.2±0.7^{b}	16.8±1.5^{ab}	2.8±0.2^{bc}
Proact®	34.0±1.1^{b}	11.3±0.8^{a}	18.1±1.0^{ab}	2.6±0.1^{c}
Abamax®	43.8±1.1^{a}	12.0±0.9^{ab}	19.1±0.8^{a}	3.9±0.1^{ab}
Cropset±Abamax	33.4±1.1^{b}	12.0±0.9^{ab}	19.5±1.1^{a}	4.0±0.4^{a}
ISR2000®±Abamax®	33.6±1.2^{b}	11.0±1.1^{ab}	16.3±0.6^{ab}	4.5±0.3^{a}
Proact®±Abamax®	33.6±1.1^{b}	13.4±0.9^{ab}	20.0±0.8^{a}	4.0±0.1^{a}
Nematode (+) Control	24.4±1.5^{c}	3.6±0.2^{c}	13.6±0.6^{b}	2.5±0.2^{c}
Nematode-free (-) Control	33.9±1.7^{b}	10.9±0.9^{ab}	15.7±1.1^{ab}	2.7±0.1^{c}

*The lowercase letters in the same column indicate statistically differences between applications (P≤0.05).

As a result of the examination of stained root, stem and leaf tissues of tomato plants, differences in lignification distribution and density were observed. Lignified structures were identified as having turned a dark pink color. The plant activator with the highest lignin staining was found to be Proact® (Figure 1), followed by ISR-2000® and Crop-Set®. In the applied plant activators, lignin accumulation was determined to be highest in leaves, stems and roots, respectively. Lignification was detected to be higher in combinations with Abamectin. The highest lignification was detected in the Abamectin and Proact combination application (Figure 2).

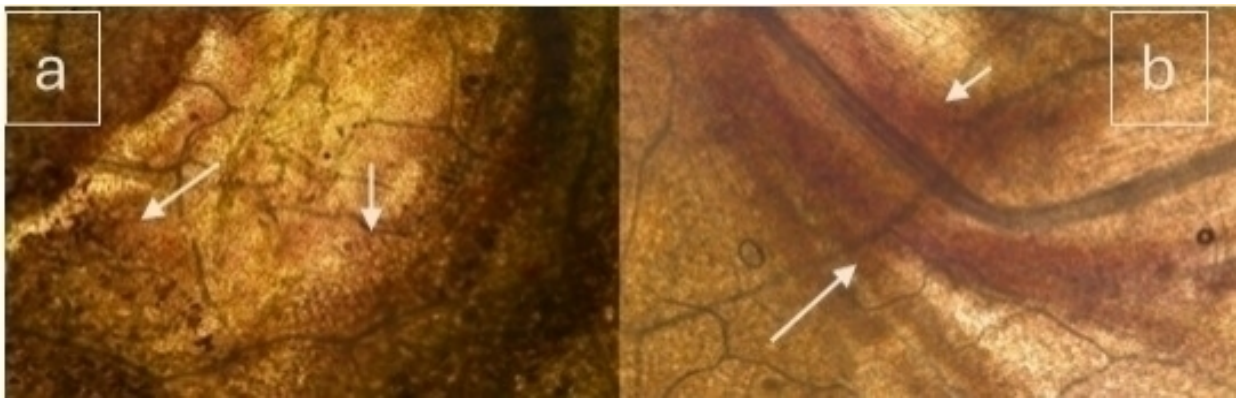

Figure 1. Lignification in the leaf (a) and stem (b) in Proact® application.

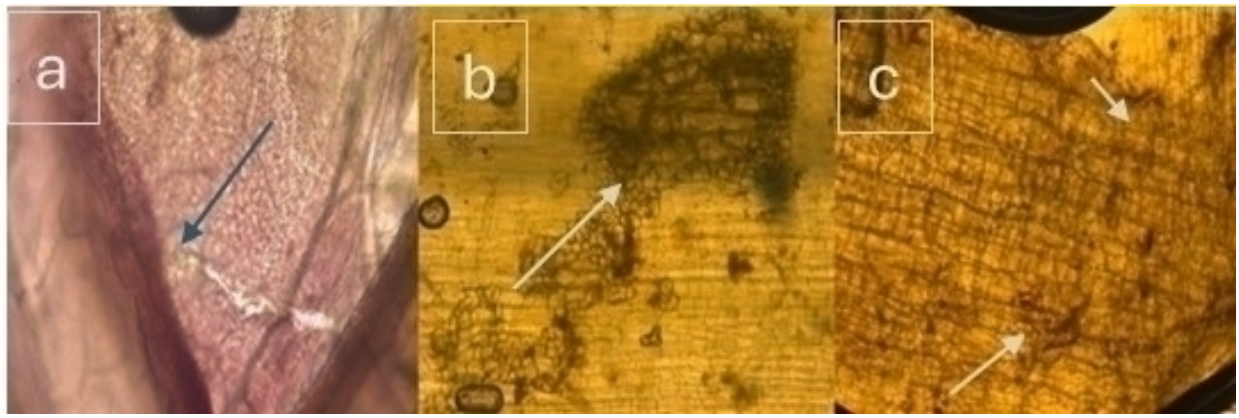

Figure 2. Lignification in the leaves (a), stems (b) and roots (c) in Abamax® and Proact® combination applications.

4. Discussion

In the study, it was determined that the application of Abamax® (abamectin) commercial preparation to the soil significantly suppressed *M. incognita* in the roots of tomatoes and had a positive effect on plant development. It is a good alternative to the fumigant methyl bromide, whose use is banned all over the world, and most carbamates and organophosphates. Abamectin is an effective nematicide recorded to control plant parasitic nematodes such as *Meloidogyne* spp., *Heterodera* spp., *Pratylenchus penetrans*, *Globodera pallida, Rotylenchulus reniformis* and *Tylenchulus semipenetrans* in different crops (Li et al., 2018; Sasanelli et al., 2020a; Massoud et al., 2023). Different studies have shown that abamectin causes an increase in plant and root system length and weight (Korayem et al., 2008; Qiao et al., 2012).

Abamectin (Vertemic 1.8% EC) has proven its nematicide effectiveness in suppressing root-knot nematodes on different vegetable crops as a soil application (Hamida et al., 2006; Khalil, 2012; Saad et al., 2012). It is stated that abamectin formulations are an important factor in biological activity against plant parasitic nematodes (Li et al., 2018). Sasanelli et al. (2020b) reported that abamectin had higher toxicity than fluopyram to the J2 of root knot nematode but the control effect of abamectin (1.8% EC, 375 g a.i./ha) was significantly lower than that of fluopyram (41.7% SC, 450 a.i./ha) in both pot and field trials. Abamectin activates glutamate-gated chloride channels, which open to allow chloride ions to enter the cell, ultimately causing hyperpolarization. This causes paralysis of the neuromuscular system and death (Cully et al., 1994). In addition, abamectin only causes sublethal toxicity in mice or other mammals at very high concentrations. However, subchronic and chronic toxic effects for low doses and long-term exposure are still unclear (Bai and Ogbourne, 2016). Low rates of accumulation over a long period of time can be highly toxic to fish and can enter the human body as part of the biological food chain (Qiu et al., 2022).

Another result of the study is that plant activators can be used in the control against root knot nematode. Although no difference was determined between plant activators in terms of gall index, a difference was found between them in terms of the number of galls and egg masses. The number of galls and egg masses in the roots was found to be lower in the Proact® (Harpin) application than in the Cropset® (*L. acidophilus*) and ISR-2000® (*L. acidophilus*) applications. As a result of dyeing studies on sections taken from the roots, stems and leaves of plants, it was determined that lignifacition increased in plants to which activator was applied and it is thought that durability was promoted. It has been shown that the continuity of resistance is ensured with fourteen-day applications. Resistance occurs as oxidative combustion, lignification or hypersensitive response when the elicitor molecules of the pathogen are recognized and activated (Chaube and Pundhir, 2005). SAR is a physiological condition that occurs with biotic or abiotic environmental stimuli that activate a plant's immune defenses (Vallad and Goodman, 2004). Plants exhibiting SAR have enhanced resistance to various pathogens. SAR can be induced by challenging a plant with lethal, harmful, and non-pathogenic microorganisms or artificially with certain chemicals (Sticher et al., 1997; Gozzo, 2003). Many compounds have been shown to be SAR elicitors, such as salicylic acid, 2,6-dichloro-isonicotinic acid (INA), benzo(1,2,3)thiadiazole-7-carbotioic acid S-methyl ester (BTH, known as acibenzolar-S-methyl) and the microbial protein harpin (Klessig et al., 2000). Collins et al. (2006) stated that BTH (acibenzolar S methyl) and harpin applications reduced the number of lesion nematodes (*Pratylenchus* spp.) in potatoes, while BTH reduced *M. chitwoodi* at the end of the season. In addition, potatoes treated with BTH and high dose harpin reduced the nematode infection index and the number of discarded potatoes by 26% compared to the control. Harpin is preferably used as foliar application and at 14-day intervals during plant vegetation. When applied to a plant, harpin proteins bind to plant receptors and stimulate many biochemical reactions through gene activation, and the resistance mechanism in the plant becomes active (Akbudak and Tezcan, 2006). Seo et al. (2019) found that *L. brevis* WiKim0069 showed the strongest nematicidal activity against the J2 of *M. incognita, M. arenaria,* and *M. hapla* in vitro. The fermentation broth of WiKim0069 also reduced gall formation on melon under field conditions, with a higher efficacy (62.8%) than that of fosthiazate (32.8%). Treatment with various *Lactobacillus* strains can also improve the innate immunity of plants through a systemic acquired resistance, resulting in the upregulation of defense-related metabolites and leading to resistance to phytopathogens (Hamed et al., 2011; Konappa et al., 2016).

In combination applications, galls and egg masses could not be found in the roots. Combination applications of abamectin and plant activators have been shown to be promising in the future, with both nematode suppression and increase in plant development. While there are studies on abamectin in combination with different chemical and biological agents, no studies were found with plant activators. Shaver et al., (2016) reported that abamectin combined with azoxystrobin had a good control effect on *Trichodorus obtusus* Cobb in Zoysia grass. The control efficacy of the combined treatment was higher than that of 1,3-D used alone and the tomato yields were increased (Qiao et al., 2014). Khalil (2013) conducted a pot trial to evaluate the effect of abamectin, *Bacillus thuringiensis* and *Bacillus subtilis*, alone or in combination, against the development of *M. incognita* in tomato plants and found that abamectin had the highest rate of reducing gall formation by 85.87%. The second most effective application was found to be abamectin ± *B. thuringiensis* (85.20%). The combination of abamectin ± *B. thuringiensis* recorded the highest increase in root length and root fresh weight. The combination of abamectin with *Purpurecillium lilacinum* and rhizobacteria was the most effective against *M. incognita*, also effective in increasing tomato plant growth parameters compared to the control (El-Ashry et al., 2021). In this study, although there was an increase in the plant and root development parameters of single applications and combination applications compared to plants with only nematode inoculation, a similarity was found between them when compared with plants without nematode inoculation and any application. No significant increase in plant and root development parameters was detected in combination applications

5. Conclusion

A root-knot nematode management strategy using plant activators will meet the world's growing demand for environmentally friendly agent that can replace synthetic chemicals and toxic pesticides in agriculture. By using abamectin once and supporting it with plant activators throughout the season, yield loss can be reduced and possible toxicological problems of abamectin can be minimized. The application of various bioagents including abamectin might be a potential antagonism strategy against root knot nematodes in protected agricultural areas. Additionally, these studies need to be supported by field studies.

Author Contributions

The percentages of the authors' contributions are presented below. All authors reviewed and approved the final version of the manuscript.

	F.G.G.	H.Ç.
C	50	50
D	50	50
S	50	50
DCP	40	60
DAI	80	20
L	20	80
W	50	50
CR	50	50
SR	40	60
PM	60	40
FA	50	50

C= concept, D= design, S= supervision, DCP= data collection and/or processing, DAI= data analysis and/or interpretation, L= literature search, W= writing, CR= critical review, SR= submission and revision, PM= project management.

Conflict of Interest

The authors declared that there is no conflict of interest.

Ethical Consideration

Ethics committee approval was not required for this study because there was no study on animals or humans.

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