Egypt. J. Agronematol., Vol. 22, No.1, PP.19-40 (2023)
DOI:10.21608/EJAJ.2023.287219

Nematicidal Activity, Oxidative Stress and Phytotoxicity of Virkon®S on Tomato Plants Infected with Root-knot Nematode

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Received: 4 October 2022 *Revised:15 February 2023* *Accepted:22 February 2023*

ABSTRACT

As a global industry, agriculture requires new chemical materials with novel properties to control stubborn pests such as soil-borne diseases. So, Virkon® S disinfectant that contains oxone (potassium peroxymonosulfate) and sodium dodecyl benzenesulfonate was tested. The study investigated Virkon S nematicidal activity against root-knot nematode (RKN), *Meloidogyne incognita*, given the lack of data. Egg hatching inhibition was more significant than 50% after one hour of egg masses exposure at a concentration of 0.4% (w/v) in Petri dishes. Virkon S can disintegrate gelatinous egg masses, generating tiny clusters that soon transform into free eggs with a high hatching reduction during embryonic development. Second stage juveniles (J2s) showed exceptional hyperactivity and excitation after less than an hour of exposure before becoming paralyzed, where 1% (w/v) concentration obtained 99.20% mortality after 12 h. Virkon's toxicity increased with concentration, followed by a short latency period, especially against eggs. Virkon S's significant nematicidal activity was evident following tomato J2s inoculation. Virkon S increased fresh root and shoot weight parameters with an increasing percentage directly proportional to applied concentrations 60 days post-application. Virkon S reduced the numbers of J2s and eggs and soil RKN reproduction at various concentrations. Additionally, all Virkon S concentrations had the least galls and egg masses/root, with the concentration of 1% having the most significant reduction in reproduction factor. Increasing the applied concentration through soil drenching led to increases in total sugar, protein, phenolic, and flavonoid content and antioxidant activity, as well as a reduction in nitrate and oxidative enzyme activities in tomato roots with no observed phytotoxic effect on plants; however, foliar spraying with concentrations greater than 0.5% (w/v) caused local leaf scorch injury, developed with 3% (w/v) concentration to plant top death and growing side buds below the damaged area. Finally, the nematicidal activity of Virkon S has been approved, but more studies are needed to eliminate its limited use in plant production.

Keywords: Virkon® S, *Meloidogyne incognita*, Nematicide, Tomato, Oxidative stress, Phytotoxicity

INTRODUCTION

Roundworms (Phylum: Nematoda) have been around for an estimated billion years, making them one of the most ancient and diverse animals on the planet (Zasada and Ferris, 2004). Nematodes have evolved extensively to inhabit vast niches, from aquatic to marine and terrestrial, present on planet earth. Soil moisture, relative humidity directly, and temperature affect nematode survival as they depend on free water for locomotion and active life cycle. Parasitism of the phylum has arisen independently across the various phylogenetic clades and infects mammalian and avian hosts (Blaxter and Koutsovoulos, 2015) and plant hosts (Sasser, 1987). Worldwide estimates for annual crop losses of plant-parasitic nematodes (PPNs) are approximately $10 billion in the USA and $125 billion globally (Sasser, 1987). According to 2011-2012 Egyptian

production figures and pricing, annual agricultural losses due to PPNs on 80 crops were estimated at 15.85 billion L.E., or about $2.30 billion (Abd-Elgawad, 2014).

Symptoms of *Meloidogyne* spp. infected plants include damage to the root system (root galls) and above-ground wilting symptoms (Collange et al., 2011). Numerous tactics are used in the management of PPNs, including nematicides that act as directly killing nematodes when used in lethal doses by hampering their reproduction. Moreover, using resistant cultivars, bioagents (fungi or bacteria), or microbial control and biotechnological approaches, such as applying nanoparticles and nanomaterials for nematode management (Subedi et al., 2020).

Searching for new powerful nematicides to replace traditional nematicides and fumigants became urgent. Novel nematicides should occur in their action on nematode with a new mechanism to surpass old nematicides also nears the fumigant's efficiency but without the need to vacate economic plants from the soil before application. Besides, the long wait period after fumigation is required to dissipate the fumigant's toxic concentration so that the land becomes suitable for growing the next crop. Mentioned obstacles put us in challenges to test untraditional new industrial substances such as Virkon® S. At a concentration of 1 % (w/v), Virkon S is a broad-spectrum disinfectant that is effective against a wide range of microorganisms, including viruses, gram-positive and gram-negative bacteria, fungi (Gasparini et al., 1995), and mycoplasma (Chao et al., 2018) that effect on global animal /plant production. Also, Virkon S is valid for nonporous hard surfaces, agricultural production equipment, vehicles, wheels, boot disinfection, and general farm sanitation. Virkon S, as a product, contains potassium monopersulphate (Oxone) and sodium dodecylbenzene sulfonate (SDBS), which have a broad spectrum of activity against viruses, some fungi and bacteria at 1% up to 3 % concentration (Carlisle, 2009; Sloan et al., 2022). Virkon S is also effective on nonporous hard surfaces, agricultural production equipment, vehicles, wheels, poultry, farm premises, disinfecting boots, and farms daily (Gabbert et al., 2020).

This study aimed to determine the nematicidal activity of Virkon® S against the deleterious soil borne nematode RKN, *Meloidogyne incognita*. *In vitro* bioassays were conducted against eggs and egg masses (ovicidal activity) and larvicidal activity against second stage juveniles (J2s mortality). The study also evaluated Virkon S soil-drenching control efficacy against *M. incognita* infected tomato plants *in vivo* as well as biochemical changes and oxidative stress associated with Virkon S application on tomato plants, in addition to its phytotoxicity to the shoot system after foliar spraying.

MATERIALS AND METHODS

1-Root-knot nematode *Meloidogyne incognita* inoculum preparation

The RKN, *M. incognita* inoculum was extracted and identified from infected tomato roots. The inoculum was maintained on susceptible tomato plant cultivar Super Strain B cultivated in sterilized pots filled with autoclaved soil under greenhouse conditions. One egg mass infected plants to establish a pure culture (Mostafa et al., 2022). The roots of infected tomato plants were used to extract nematode eggs via the sodium hypochlorite method (Hussey and Barker, 1973). The extracted nematode eggs were washed with sterilized distilled water on a sieve of 200 mesh nested upon a sieve of 500 mesh. Then eggs were collected in a sterile 50 ml Falcon and counted using a counting slide. Egg suspension was adjusted to 2000 eggs/ml for sequent *in vitro* assays. Later, the second juveniles (J2s) were obtained from free eggs by incubating egg suspension in Petri dishes at 23±3°C until hatching. The newly emerged nematode juveniles were

enumerated and prepared in 1000 J2s/ml as a stock suspension to conduct a larvicidal assay.

2. Virkon® S formulation

Virkon® S (Antec International, Sudbury, Suffolk, United Kingdom) is formulated as pink granules with a broad-spectrum disinfectant for animals used in health care. Oxone (potassium peroxymonosulfate), sodium dodecylbenzenesulfonate, and sulfamic acid are the active ingredients in the formulation.

3. *In vitro* ovicidal and larvicidal effect of Virkon S **against *Meloidogyne incognita***

Serial concentrations of 0.4, 0.6, 0.8, and 1 % (w/v) of Virkon S were prepared to evaluate *in vitro* efficacy against eggs and J2s of *M. incognita*. The dishes were incubated at 23±3°C. Petri dish was measured at 1, 3, 6, 12, 24, and 36 h post-treatment.

3.1. Ovicidal assay of Virkon S **against free eggs**

A newly extracted free egg suspension of RKN (2000 eggs/ml) was used. In 6 cm-diameter Petri dishes, a 0.1 ml volume (200 eggs/ml) was completed to 10 ml of the final volume with the concentrations mentioned earlier. The number of hatched juveniles was expressed as a cumulative number of viable J2 compared to the check control treatment, which contained only 200 eggs in 10 ml of distilled water. The percentages of hatching suppression were calculated using the following formula:

$$\text{Egg hatching inhibition (\%)} = \frac{\text{Number of hatched J2 in control} - \text{Number of hatched J2 in treatment}}{\text{Number of hatched J2 in control}} \times 100$$

3.2. Ovicidal assay of Virkon S **against egg masses**

Uniform size egg masses were picked up and transferred to 6-cm diameter Petri dishes containing 10 ml of Virkon S prepared at the concentrations mentioned and then observed at various periods. In the control plates, egg masses were mixed with 10 ml of distilled water. Treatments were incubated, and the number of hatched juveniles was counted using a research microscope (100X magnification). The cumulative number of hatched juveniles in each Petri dish was recorded. The percentage of hatching inhibition was calculated compared to the control treatment using the previous equation.

3.3. Larvicidal assay of Virkon S **against the second stage juveniles**

A 0.1 ml of newly emerged J2s suspension (1000 J2s/ml) of RKN stock suspension was transferred to a tube and complemented to a 10 ml final volume of the desired concentration of Virkon S. The final volume was poured into sterilized Petri dishes (6 cm diameter) and incubated *in vitro*. Juvenile mortalities were monitored periodically with microscope (Zeiss, Germany) at 100X magnification. The juveniles were counted as either alive or dead. The dead juveniles, which showed static straight posture and remained immotile after probing were certified dead (Ishibashi and Takii, 1993). The formula below was used to compute mortality percentages.

$$\text{Mortality (\%)} = \frac{\text{No. of dead juveniles}}{\text{Total No. of tested juveniles}} \times 100$$

Based on mortality under control, Abbott's formula (Abbott,1925) was used to correct the percentage of deaths in treatments.

4. Greenhouse experiments

4.1. Nematicidal activity of Virkon S against *M. incognita* infected tomato plants

The experiments were carried out with seedlings of tomato (*Solanum lycopersicum* L.) cv. 016 transplanted in a plastic tray. The tomato seedlings at the 3-4 leaf stage (≈10 cm length) were transplanted separately in plastic pots (12 cm deep and 17 cm in diameter). Each pot filled with 2 kg consisted of non-sterilized sandy soil. Seven days after transplanting, seedlings were inoculated with 1000 J2s of *M. incognita*, except healthy plants in control group pots. The nematode inoculum (1000 J2s) was diluted in 4 ml water using a micropipette. The diluted inoculum suspension was poured around the roots of each seedling into four holes 5 cm deep, made using a pencil, and covered immediately with wet sandy soil after inoculation. Virkon S was implemented using a soil drenching technique with 10 ml solution per plant (pot) with concentrations of 0.1, 0.5, and 1% (w/v). Two months after the application, tomato plant growth traits such as fresh weights of root and shoot (g) and leaves /plant also stem diameter (mm) were assessed. Reproduction of *M. incognita* numbers of galls /root, gall diameter classes (mm), egg masses/root, J2s/100 g soil, and eggs/100 g soil. Besides reproduction factor (RF) = Final population/ Initial population (Oostenbrink, 1968). Replicates were arranged in a completely randomized design. Nematodes were extracted from soil using a combination of sieving and the Baermann trays technique (Hooper, 1990).

4.2. Biochemical parameters

For each treatment, roots of tomato plants were separately washed with distilled water two times and drained. The plant roots were quickly cut into thin slices, weighed 5 g in a Falcon tube, and stored at -20°C. The frozen root sample was homogenized for 5 minutes at 4°C in 50 ml of 100 mM sodium phosphate buffer (pH 7.0) containing 1 mM ascorbic acid and 0.5 % (w/v) polyvinylpyrrolidone. Three layers of cheesecloth were used to remove impurities from the homogenate. The filtrate was centrifuged at 5,000 xg at room temperature for 15 minutes before collecting the supernatant to obtain the crude extract (Alici and Arabaci, 2016).

Total sugars were estimated according to Chaplin (1994) method. At the same time, Nitrates were determined according to Sharma and Kaur (2017) method. Also Follin Ciocalteu phenol reagent was used to evaluate protein concentration (Lowry et al., 1951), with bovine serum albumin serving as the reference standard. Non-enzymatic antioxidants, including Total Phenolics concentration, were measured using the Folin-Ciocalteu assay (Škerget et al., 2005). Total Flavonoids were determined based on the method described by Ordonez et al. (2006). Besides, the DPPH (1, 1-diphenyl-2-picrylhydrazyl) radical-scavenging activity was performed by Bhakya et al. (2016) to determine antioxidant activity. Antioxidative enzymes included catalase activity was measured according to the method described by Fossati et al.(1980). Besides, peroxidase activity was measured by Onsa et al. (2004) method. Both enzymes were determined using Kits supplied by Biodiagnostic, Egypt. Also, their activities were expressed as mmol of decomposed H_2O_2/min/mg protein. Besides, superoxide dismutase was assessed using the Nishikimi et al. (1972) method. The enzyme activity was expressed as mmol superoxide/min/mg protein.

4.3. Virkon S phytotoxicity

Virkon S spray solutions of 0, 0.5, 1, 1.5, 2, and 3% (w/v) concentrations were used. Each tomato plant was sprayed with 10 ml of spray solution, evenly distributed throughout, using a hand-held sprayer's nozzle and pump. Plants were monitored for developing severe phytotoxicity symptoms for six consecutive days after treatment.

Statistical analysis:

The experiments' data were subjected to variance analysis (ANOVA) using MSTAT version 4. Means were compared using Duncan's multiple range test at a significance level of $p \leq 0.05$. Biochemical parameters are expressed as the mean ±SE of three independent measurements to determine statistical significance.

RESULTS AND DISCUSSION

Nematicidal activities of Virkon S against *M. incognita* egg masses, eggs and second stage juveniles

The ideal disinfectant is distinguished by the consistency of disinfection speed, the concentration coefficient, and the minimal inhibitory concentration (Gasparini et al., 1995). Based on laboratory observations on Virkon S after an hour of exposure, a high egg hatching inhibition percent exhibited with the lowest concentration (0.4 %) exceeded 50% to reach 78.79% after six hours. Increasing exposure time to 24 h incubation increased potency to nearly 96.96%.

During the experiment, the percentages of non-hatching eggs exposed to Virkon S always stopped egg hatchability exceeding 90% after one-hour exposure with 0.6, 0.8 and 1.0 % concentrations compared to the control treatment (distilled water). The data trend was repeated, with no significant difference with more extended exposure periods (Table 1). It was observed that the tested concentrations could stop egg hatch at all embryonic egg development. Virkon S toxicity increased gradually with increasing the tested concentrations and time exposure; reaching 1% concentration showed the maximum effect against egg masses of *M. incognita in vitro*.

Table 1: Virkon's ovicidal activity against *Meloidogyne incognita* egg masses.

Virkon S Concentrations (%; w/v)	No. of emerged juveniles and egg hatching reduction (%) after exposure periods (h)					
	1	3	6	12	24	36
0	4.40^{a}	10.80^{a}	19.80^{a}	46.80^{a}	98.80^{a}	199.20^{a}
0.4	2.00^{b} (54.55)	4.80^{b} (55.56)	4.20^{b} (78.79)	1.20^{b} (97.44)	3.00^{b} (96.96)	5.00^{b} (97.49)
0.6	0.40^{c} (90.91)	1.60^{c} (85.19)	1.00^{c} (94.95)	1.20^{b} (97.44)	1.80^{bc} (98.18)	3.80^{b} (98.09)
0.8	0.40^{c} (90.91)	1.00^{c} (90.74)	0.80^{c} (95.96)	1.20^{b} (97.44)	1.40^{bc} (98.58)	2.80^{b} (98.59)
1	0.40^{c} (90.91)	0.40^{c} (96.30)	0.80^{c} (95.96)	0.80^{b} (98.29)	1.20^{bc} (98.79)	1.20^{b} (99.40)

*Reported numbers represent the means of 5 replicates.

**Figures in parentheses are egg hatching reduction (%) compared to control.

*** Significant variances are indicated by different letters in the same column ($P \leq 0.05$) using Duncan's multiple range test.

The toxicity of Virkon S against free eggs of *M. incognita* was evaluated, as shown in Table (2). All the tested concentrations could reduce the hatching of *M. incognita* eggs. The concentration of 0.4% stopped hatchability after 24 h recorded at 89.58 %. The percentage of egg hatching inhibition was 75.00 and 87.50 % after one-hour exposure with concentrations of 0.4 and 0.6%, causing hatching reduction of 91.18 and 94.12% after 12 h, respectively

Table 2: Virkon's ovicidal activity against *Meloidogyne incognita* eggs.

Virkon S Concentrations (%; w/v)	No. of emerged juveniles and egg hatching reduction (%) after exposure periods (h)					
	1	3	6	12	24	36
0	1.60[a]	2.20[a]	4.00[a]	6.80[a]	9.60[a]	13.00[a]
0.4	0.40[b] (75.00)	0.40[b] (81.82)	0.40[b] (90.00)	0.60[b] (91.18)	1.00[b] (89.58)	2.40[b] (81.54)
0.6	0.20[b] (87.50)	0.40[b] (81.82)	0.40[b] (90.00)	0.40[b] (94.12)	0.80[bc] (91.67)	1.80[b] (86.15)
0.8	0.20[b] (87.50)	0.40[b] (81.82)	0.40[b] (90.00)	0.40[b] (94.12)	0.80[bc] (91.67)	1.40[bc] (89.23)
1	0.20[b] (87.50)	0.20[b] (90.91)	0.20[b] (95.00)	0.40[b] (94.12)	0.60[bc] (93.75)	0.60[c] (95.38)

*Reported numbers represent the means of 5 replicates.
**Figures in parentheses are percentages of egg hatching inhibition compared to control.
*** Significant variances are indicated by different letters in the same column ($P \leq 0.05$) using Duncan's multiple range test.

Also, all the tested concentrations could reduce the hatchability of *M. incognita* eggs at all different embryonic development stages and varied according to Virkon S concentrations. Moreover, the emerged J2s after only one-hour exposure were immotile and paralyzed in a straight posture to death entirely after three hours of hatching. Generally, concentrations and exposure time are crucial factors in stopping egg hatching. *Meloidogyne incognita* J2s, an infective stage, causes a severe loss to the host plants, and the severity increases with an increase in inoculum density. The high toxicity of Virkon S concentrations after a different exposure time against juveniles is shown in Table (3). *In vitro*, the J2s mortality assay for *M. incognita* revealed that the percentage of mortality exceeded 50% after 12 h of exposure at 0.4% concentration and increased to 83.40% after 36 h. After one hour of exposure to Virkon S concentrations of 0.6 and 0.8%, the death rate of J2s increased from 27.80 to 47.60%. The concentration of 0.8% gradually increased from 47.60 to 83.80 % after one and six hours, respectively.

On the other hand, J2s mortality percent was recorded at 49.20 and 85% after only one and three hours, respectively. The results emphasize that the killing rate is directly proportional to the Virkon S amount and time of J2s exposure. Furthermore, after one hour of exposure, J2s were immotile, paralyzed in a straight posture, and died after three hours.

Concerning Virkon S ovicidal effect, microscopic examination showed several changes in a brief latent period. These changes included dissolving the gelatinous matrix coating egg masses of the RKN, causing egg dispersal in single eggs or small clusters that did not exceed 3 to 4 eggs (Fig.1). Regarding eggs, the color changed from shiny to colorless gradually with slowing movement step by step, then stopped movement and dead in little time with the continued increase in the eggs' luster even after the death of the internal embryos and juveniles. The complete development egg stage contained complete J2, failed to hatch, and was utterly dead with the second juvenile inside it (Fig.1 & Fig.2) and with the same observation with other embryo egg

Table 3: Virkon's larvicidal activity against *Meloidogyne incognita* second stage juveniles.

Virkon S Concentrations (%; w/v)	No. of dead juveniles and mortality (%) after exposure periods (h)					
	1	3	6	12	24	36
0.4	38.00^{b} (19.00)	81.60^{d} (30.80)	88.80^{d} (44.40)	118.40^{c} (59.20)	136.40^{c} (68.20)	166.80^{b} (83.40)
0.6	55.60^{b} (27.80)	78.00^{c} (39.00)	118.00^{c} (59.00)	167.60^{b} (83.80)	192.40^{b} (96.20)	200^{a} (100)
0.8	95.20^{a} (47.60)	115.60^{b} (57.80)	167.60^{b} (83.80)	188.40^{a} (94.20)	198.40^{a} (99.20)	200^{a} (100)
1	98.40^{a} (49.20)	170.00^{a} (85.00)	190.80^{a} (95.40)	198.40^{a} (99.20)	200^{a} (100)	200^{a} (100)

*Reported numbers represent the means of 5 replicates.
** Figures in parentheses are corrected juvenile mortality percentages based on the death rates in the control group using Abbott's equation.
*** Significant variances are indicated by different letters in the same column ($P \leq 0.05$) using Duncan's multiple range test.

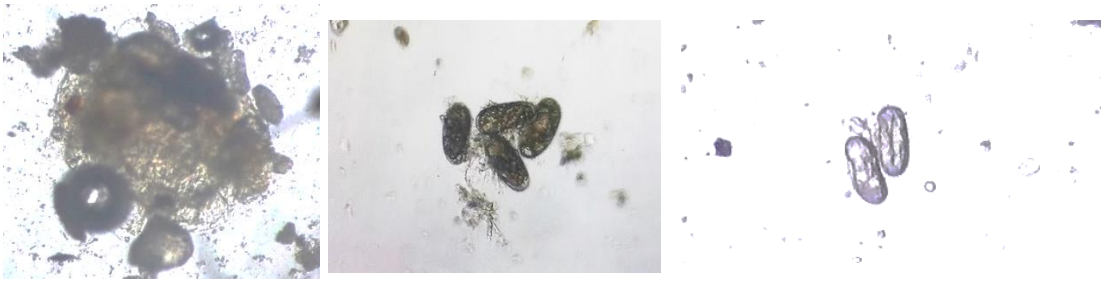

Figure 1: Eggs of single egg mass coated with gelatinous matrix in *Meloidogyne incognita* (untreated control) at various stages of embryonic development (left); Virkon S could dissolve gelatinous matrix which coating RKN egg masses, resulting in small clusters of RKN eggs (3 - 4 eggs; middle); changes in eggs morphological aspects and stop development (right).

Figure 2: *Meloidogyne incognita* free eggs at various stages of embryonic development (left) in untreated control, dead eggs (middle) with abnormal morphological aspects, dead eggs contain prehatching J2 inside egg shell and dead egg with eggshell decay showed egg content migration (right).

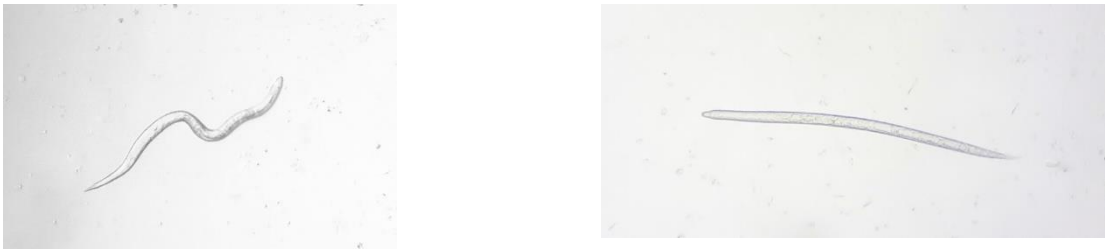

Figure 3: Live second infective stage (J2) of *M. incognita* actively moving in distilled water of control treatment (left) and straight dead J2 in Virkon S treatment (right).

development with lysis eggshell and egg content flow (Fig.2). At the beginning of exposure, it was observed that some juveniles hatched successfully from a few eggs after a brief period of up to 15 minutes and showed unusual hyperactivity and excitation. After an hour from hatching, J2 die gradually and are fixed in straight shapes or other shapes, with a gradual change in color to become more transparent or shiny and reach complete transparency after their death (Fig.3). This action causes effective egg hatching reduction with a short latent period within about an hour of exposure.

The larvicidal effect of Virkon S was substantial on emerged J2. For example, after 10 minutes of J2 exposure, hyperactivity and excitation were observed compared to J2 in the control. After 25 minutes of incubation, the juveniles' movements slowed down gradually with the shining juvenile viscous, and then J2 died thoroughly after half an hour. By the time after death, the luster of the body of the J2 increases (Fig.3). By looking at the severity of the symptoms under a microscope, it was noted that the poisoning was strongly positively correlated with the applied concentration, and the Virkon S concentration was strongly negatively correlated with the latency period, followed by the period between treatments and symptoms observation. As the egg masses of RKN gradually disappeared, they turned into free eggs in the solution. Vanishing egg masses required a more extended incubation period with lower applied concentration. Virkon S ovicidal effect was practical in different embryonic development stages. The extension of incubation caused the eggshell erosion and flux of its content or the egg's death during the embryonic development stage upon treatment.

Virkon S contains potassium monopersulfate (KMPS), sodium dodecylbenzene sulfonate (SDBS), sulfamic acid (SA), and inorganic buffers as a multi-purpose disinfectant. The KMPS acts as an oxidizing agent. Accordingly, SA was added to produce a low pH, which is responsible for increasing the potency of Virkon S. An inorganic buffer (sodium hexametaphosphate) was incorporated to stabilize these acid conditions. The surfactant called SDBS was added to combine cleaning and disinfection. Virkon S contains additional components, e.g., sodium chloride salt (NaCl) and pink dye.

The complex chemical pathway of Virkon's action, the Haber-Will-Statter Reaction, includes sodium chloride, which forms chlorine gas oxidized by KMPS. It interacts with the SA (acting as a chlorine acceptor) to create an intermediary complex. It hydrolyzed this complex to release hypochlorous acid (HOCl) as a potent biocide (Jones and Joshi, 2021). The chloride emitted by SA is directed towards producing more sodium chloride, refueling the cyclic system (Lorenzi et al., 2008). The pink dye is a built-in color indicator of biocidal activity. It is pink in its oxidized form but turns colorless when the solution loses its activity. If a Virkon S solution's color begins to fade, it must always be replaced as directed on the label. This proposed mechanism may

be responsible for the Virkon S broad-spectrum against mentioned numerous microorganisms and safety for humanity. As a result of non-selectivity against microbes caused by multi-components, optimized, an oxidizing system of Virkon S that would destroy all organisms, the maximum solubility dose of Virkon S is 4%, which would be avoided due to misapplication.

Virkon S toxicity mechanism is similar to the chorine mechanism applied in nematode extraction (Eisenback, 2000). Chlorine can dissolve gelatinous matrix-coated egg masses (Adam et al., 2014) and release nematode egg using sodium hypochlorite solution 0.5% (w/v) with 5 min exposure. The use of chlorine in the sterilization of water-related diseases to abate health diseases, thereby ensuring the populace's health safety (Okpara et al., 2011). The use of chlorine is commonly used to disinfect potable water and wastewater. Typically, chloride-based disinfectants, such as chlorine, chlorine dioxide, and hypochlorite salts, are utilized. The ion hypochlorite (OCl^-) is far less effective than chlorine and hypochlorous acid (HOCl) (Margerum et al., 1979).

Several factors that affect the effectiveness of chlorine disinfection, including the water's pH and turbidity, as well as its chlorine concentration and contact time. When introduced to water, chlorine rapidly hydrolyses, releasing HOCl and hydrochloric acid. Hydrolysis to HOCl is nearly complete at pH values greater than four and chlorine concentrations up to 100 mg/L. The weak acid hypochlorous acid partially dissociates into a hypochlorite ion ($OC1^-$). At pH 7.5, HOCl and $OC1^-$ are equally distributed; at pH 6.5, 90% of the free chlorine is present as HOCl; and at pH values more than 9, hypochlorite ions constitute the significant species. Hypochlorous acid is a significantly more effective disinfectant than hypochlorite ion. Hence a lower pH promotes effective disinfection (Dychdala, 2001; Galal-Gorchev, 1996).

Virkon acidifying the water tends to encourage the creation of hypochlorous acid, causing increasing chlorine's effectiveness. It is crucial not to combine chlorine and acids, as doing so produces poisonous chlorine gas (Fukuzaki, 2006). HOCl is a rapid-acting and antibacterial solid agent that interacts with multiple biomolecules, including sulfur-containing amino acids, lipids, nucleic acids, and membrane components, resulting in severe cellular damage. Additionally, it oxidizes sulfhydryl groups, damages iron-sulfur centers, deactivates nutrient transport, impedes cell respiration, and diminishes the ability of cells to maintain enough adenylate energy charge to be viable (Ghernaout, 2017).

Based on the explanation provided above for conventional chloride-based disinfectant compounds, Virkon's storage and transportation capabilities are superior. It is safe because the solution is non-irritant to the skin and eyes, so no elaborate protective clothing is needed. Because it does not have a harmful vapour phase, expensive ventilation is not required. Furthermore, it is compatible with all materials. Virkon's only flaw is that it is highly susceptible to hydration during improper storage, decreasing effectiveness. The obtained results clearly showed that the treatments of Virkon S concentrations drastically reduced *M. incognita* reproduction proving their efficacy as chemical control treatments.

Virkon S nematicidal effect on *M. incognita* reproduction and tomato plant development

A sedentary endoparasitic nematode, *M. incognita*, causes a tremendous severity loss in infected tomato plants compared with healthy plants and those treated with three concentrations of Virkon S (Table 4). Fresh root and shoot weight parameters provide a rapid glance at the damage ratio incurred due to the parasite of J2s of *M. incognita* and achieved enhancement by application of Virkon S. For instance, the results exhibited an increase in fresh plant biomass (root and shoot weight) reached 9.28, 51.12

and 58.15 % in roots treated with 0.1, 0.5, and 1.0% of Virkon S, respectively, and the parallel percentages in shoot weight were 33.33, 48.15 and 51.85 % applied with 0.1, 0.5 and 1.0% of Virkon S, respectively.

The rate of growth increase in leaves weight and stem diameter were also found to be in keeping with Virkon S concentrations, as displayed in Table (4), which emphasizes that the percentage of increase is directly proportional to applied concentrations. Leaves weight (6.35& 15.87%) and stem diameter (36.59 & 56.10%) displayed the most significant effect of 0.5 and 1.0 % concentrations 60 days post application.

Table 4: Effect of Virkon S on growth characteristics of tomato plants infected with *Meloidogyne incognita*.

Treatments	Tomato growth Parameters			
	Fresh weight (g)			Stem diameter (mm)
	Root	Shoot	Leaves /plant	
Healthy plants	27.825^{a}	23.00^{a}	19.50^{a}	6.80^{a}
Infected plants	10.688^{c}	13.50^{d}	15.750^{d}	4.10^{c}
Infected plants + 0.1% (w/v) Virkon S	11.680^{c} (9.28)	18.00^{c} (33.33)	16.50^{c} (4.76)	5.20^{b} (26.83)
Infected plants + 0.5% (w/v) Virkon S	16.152^{b} (51.12)	20.00^{b} (48.15)	16.75^{c} (6.35)	5.60^{b} (36.59)
Infected plants + 1% (w/v) Virkon S	16.903^{b} (58.15)	20.50^{b} (51.85)	18.25^{b} (15.87)	6.40^{ab} (56.10)

*Figures presented in parentheses in Virkon S treatments are the increasing percent of plant growth values (attributed to infected tomato plants).

** Means in each column with different superscript letters differ significantly (P >0.05) using Duncan's multiple range test.

Virkon's nematicidal efficacy against *M. incognita* under greenhouse conditions, measured by the multiplication of *M. incognita* inside the tomato roots, number of galls, and gall size, gave a reasonable estimation of disease severity caused by *M. incognita*. The application of the various concentrations of Virkon S significantly decreased the population density of *M. incognita* parameters (number of J2s and eggs) and RKN reproduction compared to non-treated inoculated plants. The treatments of Virkon S significantly ($P<0.05$) showed that tested concentrations counteract the negative impact of the nematode entirely and reduce RKN population in pot soil and galls/root (Table 5).

The maximum number of root galls exhibited in infected plants reached 98.40 compared with 20.20, 15.80, and 10.40 in roots of tomato plants treated with 0.1, 0.5, and 1.0% of Virkon S, respectively. On the other hand, none of the gall size (≥4 mm) was recorded in the roots of tomatoes plants treated with Virkon S with the least number of gall size (2 mm to < 4mm). Moreover, most minor egg masses/roots were achieved in tomato plants treated with 0.1, 0.5, and 1.0% of Virkon S

Table 5: Virkon S nematicidal efficacy against *Meloidogyne incognita* infectivity and reproduction in the tomato plant**.**

Treatments	Galls and egg masses				Population density		
	No. of galls /root	Galls size classification (mm)			No. of egg masses /root	No. of RKN stages/100 g soil	
		< 2	2 to <4	≥4		J2s	Eggs
Infected plants	98.40[a]	21.40[a]	63.00[a]	4.00[a]	73.00[a]	113.50[c]	46.82[a]
Infected plants + 0.1%(w/v) Virkon S	20.20[b] (79.47)	15.80[b] (26.17)	4.40[b] (93.02)	0.00 [b] (100)	54.25[b] (25.68)	32.60[a] (71.28)	8.37[bc] (82.12)
Infected plants + 0.5%(w/v) Virkon S	15.80[b] (83.94)	7.80[bc] (63.55)	2.60[c] (95.87)	0.00[b] (100)	42.25[c] (42.12)	20.00[b] (82.38)	9.11[b] (80.54)
Infected plants + 1%(w/v) Virkon S	10.40[c] (89.43)	9.60[bc] (55.14)	5.20[b] (91.75)	0.00[b] (100)	30.75[d] (57.88)	18.00[bc] (84.14)	6.77[bc] (85.54)

*Figures presented in parentheses in Virkon S are the reduction percent of *Meloidogyne incognita* (attributed to infected tomato plants).
** Means in each column with different superscript letters differ significantly (P > 0.05) using Duncan's multiple range test.

Table 6: The effectiveness of Virkon S in controlling *Meloidogyne incognita* reproduction on tomato roots.

Treatments	*Meloidogyne incognita* Reproduction		
	Final Population	Reproduction factor	Reduction (%)
Infected plants	2805.3	2.8053	00.00
Infected plants +0.1%(w/v) Virkon S	716.9	0.7169	74.44
Infected plants +0.5%(w/v) Virkon S	509.4	0.5094	81.84
Infected plants +1%(w/v) Virkon S	433.4	0.4334	84.55

*Initial population of *Meloidogyne incognita* at the time of transplanting was 57.142 J2/100 cc soil; Reproductive factor (RF) = Pf/Pi, where Pf -Final population, and Pi - initial population.

Although tomato plants were susceptible to infection by *M. incognita*, data supports the efficacy of Virkon S in reducing *M. incognita* reproduction. In terms of the reproduction factor (RF), treatments involving three concentrations of Virkon S as nematicide were found to be most effective, resulting in the most minor final population counts (Final population= No. J2/100g soil +No. eggs/100g soil/Initial population) in the soil of pots. Maximum reduction in RF was achieved with infected tomato plants treated with 1.0% of Virkon S (84.55%), followed by 81.84 and 74.44% in plants treated with 0.5 and 0.1% Virkon S, respectively (Table 6). In irrigation systems, water treatment methods are utilized to reduce biological problems such as algae, biofilm blockage of irrigation lines, and infections. Chlorination is an effective treatment option with minimal installation and operation expenses. However, growers need help determining the correct chlorine dose for treating their problem without causing phytotoxicity in crop plants. (Raudales et al., 2011).

The obtained results clearly showed that the treatments of Virkon S concentrations drastically reduced *M. incognita* reproduction proving their efficacy as chemical control treatments. But comparing with the conventional chloride-based disinfectants where cysts of the potato cyst nematode disintegrate in sodium hypochlorite (1% accessible chlorine). The exposure of *M. javanica* eggs to 50 or 125 mg of available chlorine/ml for more than one hour decreased hatching the following week. When J2s were exposed to >2 g accessible chlorine/ml for >24 hours, their motility and ability to generate galls were eliminated (Wood and Foot, 1975). A study that applied sodium hypochlorite to irrigation water and hydroponic growing media to control RKN (*M. javanica*) concluded that the nematode's hatch, motility, and infectivity were not affected (Stanton and O'Donnell, 1994), Perhaps the concentration of the applied disinfectant was low in order to prevent negative effects on growing plants.

Biochemical changes and antioxidative indices in tomato roots treated with disinfectant Virkon S

Stress is an external element that hurts plants (Lazar, 2003). Numerous stress events and variables occur during a plant's life cycle, either natural or artificial stressors (Lichtenthaler, 1996). Also, it may be categorized as biotic (infection or competition by other species) and abiotic stressors (physicochemical factors such as temperature, water, radiation, chemicals, Etc.). Chemical stress may be resulted from agrochemicals such as pesticides inducing biochemical changes inside plants. Stress can be induced by altering and desynchronizing oscillations of biochemical and physiological processes, resulting in a reduced state of plant resistance. Excess formation of active oxygen species such as superoxide anion, hydrogen peroxide, and hydroxyl radicals, which

causes "oxidative stress," is one of the primary processes whereby plants are destroyed. Syntoxic stimuli induce a state of passive tolerance, allowing for peaceful coexistence with stressors. In contrast, catatoxic stimuli primarily act by producing "detoxifying" enzymes, free radical scavengers, antioxidants, and other compounds that aggressively assault the stressor (Jansen and Potters, 2017). Antioxidants can help protect against free radicals detrimental effects by neutralizing them and preventing them from causing damage (Nimse and Pal, 2015).

The effect of three Virkon S concentrations [0.1, 0.5, and 1 % (w/v)] were bioassayed after being applied on tomato seedlings infected with *M. incognita* on sugars, nitrate, phenolic, flavonoid, and total protein contents in roots (Table 7). It is evident that the total sugar increased gradually in the roots of tomato infected with RKN, *M. incognita* treated with Virkon S concentrations and was higher than those in healthy plants and infected roots plants non-treated with Virkon S. The lowest total sugars amount was found in infected roots plants non-treated with Virkon S (653.91 mg/L) followed by health plants (680.40 mg/L). In contrast, the maximum total sugars amount was found in the roots of infected plants treated with Virkon S 0.1 % (694.73 mg/L), followed by 0.5 % (760.20 mg/L) and 1 % (786.36 mg/L), respectively.

Total sugars in plants, including monosaccharides, disaccharides, and polysaccharides (such as starch cellulose and glycogen), are essential for the plant's energy metabolism and serve as precursors for synthesizing structural components such as cell walls and storage compounds like starch. The levels of total sugars in plants during periods of active growth and photosynthesis, total sugar levels may be high as a result of the plant's ability to convert light energy into chemical energy in the form of sugars. On the other hand, the plant may decrease its sugar levels to allocate energy to survival under stress conditions such as drought or disease. A decrease in sucrose and starch content was observed in short-term stress experiments. In long-term experiments, a higher amount of soluble sugars and a lower amount of starch were found under stress (Gaur and Sharma, 2013).

Nitrate is one of the primary forms of nitrogen that plants take up from the soil and are used to make amino acids, proteins, and nucleic acids, which are essential for plant growth and development. Nitrate ions (NO_3^-) are usually taken up by the root system and transported to the leaves. Nitrate amount could indicate healthy root system function, especially with the application of Virkon S on tomato roots. Virkon S approved a non-systemic and non-selective phytotoxic effect on the plant. Reduction in Nitrate uptake amount approves non-observed damage in root with Virkon lower concentrations. Nitrate content was correlated negatively with protein content which may be resulted from protein denaturation resulting in Virkon S toxicity.

On the other hand, total protein varied greatly to score the minimum concentration in non-treated tomato plants (777.63 mg/L), followed by healthy plants (819.46 mg/L). In contrast, the maximum total protein (mg/L) concentration was obtained from roots of infected plants + 1% (w/v) Virkon S (951.77 mg/L) then decreased gradually with infected plants + 0.5 % (w/v) Virkon S to reach 909.33 and 845.68 mg/L with 0.1 % (w/v) Virkon S. The increasing total protein content result from the toxic mechanism Virkon S to denature the active protein losing structure and function. Thus, the situation forces the cells to amend active proteins, increasing nitrate demand, decreasing nitrate content, and increasing total protein.

Table 7: Effect of Virkon S applied to tomato plants infected with *Meloidogyne incognita* on sugars, nitrate, phenolic, flavonoid and total protein contents in roots.

Treatments	Total sugars (mg/L)	Nitrate (mg/L)	Phenolic content (mg/L)	Flavonoids content (mg/L)	Total protein (mg/L)
Healthy plants	680.40±0.23^{d}	21.31±0.11^{b}	6534.00±20.78^{bc}	1168.00±3.16^{d}	819.46±0.31^{d}
Infected plants	653.91±0.63^{e}	20.18±0.40^{c}	6254.50±26.27^{c}	1120.00±5.39^{e}	777.63±4.41^{e}
Infected plants +0.1 % (w/v) Virkon S	694.73±2.73^{ac}	22.46±0.02^{a}	6626.00±31.59^{b}	1200.00±6.78^{c}	845.68±2.50^{c}
Infected plants +0.5 % (w/v) Virkon S	760.20±2.77^{b}	20.55±0.26^{bc}	7295.50±23.18^{a}	1296.00±5.85^{b}	909.33±0.39^{b}
Infected plants +1 % (w/v) Virkon S	786.36±3.67^{a}	18.65±0.20^{d}	7440.00±21.24^{a}	1360.00±7.53^{a}	951.77±1.02^{a}

Data are present as means ± standard error (SE); superscripts a, b, and c represent a significant difference ($p \leq 0.01$) using Duncan's multiple range test.

The decrease in the enzymatic activity of the treatments proved this assumption via increasing protein content despite the decrease in the enzymatic activity of the treatments.

Concerning nitrate content, infected tomato roots treated with Virkon S concentration of 0.1 % displayed high (22.46 mg/L) significant differences ($p \leq 0.01$) of nitrate content with non-significant differences between healthy (21.31 mg/L). The treated tomato plants with 0.5% Virkon S (20.55 mg/L). The minor nitrate concentration was found in the roots of tomatoes treated with Virkon S concentrations of 1 % (18.65 mg/L).

Phytochemicals, including flavonoids, tannins, and phenolic acids, are non-enzymatic defense techniques, while enzymatic techniques include catalase, peroxidase, superoxide dismutase, and polyphenol oxidase (Khajuria and Ohri, 2020). This system allows for the scavenging of reactive oxygen species, which protects plant cells from oxidative damage (Jaleel et al., 2008; Saffar et al., 2009). These biomolecules are known to protect plants against oxidative stress and other environmental challenges.

Antioxidant enzymes can stabilize or deactivate free radicals before they oxidize cell components. There are several routes for antioxidant enzyme preservation; they could reduce free radical energy or give up some of their electrons for utilization, causing them to become stable. Furthermore, they may disrupt the oxidizing chain reaction to reduce the damage produced by free radicals (Alici and Arabaci, 2016).

Phenolic chemicals are a vast collection of secondary metabolites found in plants that have antioxidant effects. Flavonoids, tannins, and lignans are examples of phenolic compounds. High phenolic and flavonoid levels are generally regarded as indicators of high antioxidant activity and play a role in the plant defense system (Zhao et al., 2017). With phenolic content, all tomato plants treated with three Virkon S concentrations displayed higher phenolic contents than infected non-treated and healthy plants. Moreover, high phenolic content was obtained from the roots of tomatoes plants treated with 1 % (7440 mg/L) and 0.5 % (7295.50 mg/L), respectively. A maximum flavonoids content was assessed in roots treated with 1 % (1360.00), followed by 0.5 % (1296 mg/L) and 0.1 % (1200 mg/L) Virkon S, whereas the minor flavonoids content (mg/L) was assessed in roots of non-treated infected plants (1120 mg/L).

It could be concluded that total sugar, phenolic, flavonoids and total protein content were higher in the roots of tomato plants treated with Virkon S concentrations than in infected, non-treated and healthy tomato plants. At the same time, high nitrate content was only exhibited in tomato roots treated with 0.1 % (w/v) Virkon S concentration and exceeded the healthy and infected non-treated plants.

Catalase, peroxidase, superoxide dismutase, and antioxidant activity were assessed in tomato roots treated with Virkon S concentrations. Data displayed in Table (8) showed a significant influence on the catalase indices of tomato roots. Increasing the applied concentration of Virkon S results in a significant reduction in the activity of oxidative enzymes compared to lower concentrations of Virkon S, confirming the compound's ability to generate high levels of free radicals. In response, the plant increases the enzymatic activity responsible for detoxifying these radicals, neutralizing them more than the plant’s ability to inhibit the plant's detoxication enzymes. Catalase is an enzyme in plant cells that catalyzes the decomposition of hydrogen peroxide into water and oxygen (Andrade Júnior et al., 2005). Hydrogen peroxide is a poisonous result of metabolic processes in the cell, and if it were allowed to build, it would damage or kill the plant (Anjum et al., 2016). This enzyme is essential because hydrogen peroxide is a hazardous byproduct of specific metabolic processes. Catalase is a preventive agent that neutralizes hydrogen peroxide before it can cause damage (Engwa, 2018).

Peroxidase is a class of enzymes that catalyzes the oxidation of different substrates utilizing hydrogen peroxide as the oxidant (Pandey et al., 2017). Peroxidases have a range of activities in plants, including aiding in forming lignin in cell walls, toxic detoxifying substances, and contributing to plant defense systems against pathogens (Gall et al., 2015). Superoxide dismutase is an enzyme that catalyzes the transformation of superoxide ($O_2^{\bullet -}$) into oxygen (O_2) and hydrogen peroxide (H_2O_2). Copper, zinc, and manganese are the metal ions found at the active site of plants' three different forms of superoxide dismutase (Wang et al., 2016). Each kind has distinct characteristics, subcellular localization, and involvement in the plant's response to various stress conditions (Gill et al., 2015). These enzymes serve as plant defenders, neutralizing superoxide before it can cause damage. SOD deficiency has been associated with various health issues, including an increased risk of oxidative stress.

Concerning peroxidase, maximum peroxidase activity was assessed in tomato roots treated with 0.1 % Virkon S (0.75), followed by 0.5 % (0.66) and 1 % (0.56). The same trend was observed with superoxide dismutase, with insignificant differences ($p \leq 0.01$) between healthy and non-treated infected roots.

Table 8: Effect of Virkon S applied to tomato plants infected with *Meloidogyne incognita* on antioxidative indices in roots.

Treatments	Catalase	Peroxidase	Superoxide dismutase	Antioxidant activity (%)
Healthy plants	0.82±0.01^{b}	0.69±0.01^{b}	1.68±0.05^{b}	73.30±0.17^{d}
Infected plants	0.83±0.01^{b}	0.68±0.01^{b}	1.62±0.07^{b}	71.00±0.58^{e}
Infected plants + 0.1 % (w/v) Virkon S	0.88±0.02^{a}	0.75±0.01^{a}	1.88±0.01^{a}	76.00±0.58^{c}
Infected plants + 0.5 % (w/v) Virkon S	0.78±0.01^{c}	0.66±0.02^{b}	1.69±0.01^{b}	80.50±0.29^{b}
Infected plants + 1 % (w/v) Virkon S	0.70±0.01^{d}	0.56±0.00^{c}	1.56±0.02^{b}	86.00±0.58^{a}

Data are present as means ± standard error (SE); superscripts a, b, and c represent a significant difference ($p \leq 0.01$).

Treated tomato plants with [(0.1 %, 0.5 %, and 1 % (w/v)] of Virkon S displayed higher antioxidant activity (%) than healthy and infected non-treated plants. Also, antioxidant activity (%) increased gradually with an increase in Virkon S concentrations, as shown in Table (8). Antioxidant activity (%) could be arranged gradually from lowest to high as follows: 71.00, 73.30, 76.00, 80.50, and 86.00 % with infected plants, healthy plants, infected plants + 0.1 % (w/v) Virkon S, infected plants + 0.5 % (w/v) Virkon S and 1 % (w/v**)** Virkon S, respectively. Detoxification of reactive oxygen species is one of the requirements for aerobic existence, and the various lines of defense that have developed into antioxidant defense systems are veritable antioxidant defense systems. Loss of osmotic responsiveness, wilting, and necrosis result when a plant's capacity to scavenge oxygen-free radicals surpasses its capacity to produce them. Membrane breakdown and protein aggregation are both subcellular symptoms. Consequently, maintaining a balance between producing and removing activated oxygen species is crucial for healthy development, metabolism, and environmental adaptation (Hussain et al., 2022; Leshem and Kuiper, 1996). In general,

minimum activities of catalase, peroxidase, and superoxide dismutase were obtained with tomato plants treated with 0.1 % (w/v) Virkon S. Although, maximum total protein content was obtained from roots treated with 1 % (w/v) Virkon S and vice versa with the concentration of 0.1 % (w/v) Virkon S.

Phytotoxic effect of Virkon S following foliar spray

On a tomato plant in the fruiting stage, the phytotoxic effect of Virkon S on the plant's shoot system was examined. As a healthy control, the lowest concentration (0.5% (w/v) Virkon S) exhibited no observable deleterious effects (Fig. 4). As the concentration of Virkon S increased, the symptoms of toxicity began to appear on the intercalary and secondary leaflets without affecting other parts of the vegetative system, beginning with the death of the leaflets, and yellowing of some leaves at the concentration of 1%(w/v), followed by the death of some parts of the edges of the large leaves. As the concentrations of Virkon S increased, so did the severity of the symptoms and the area of the leaves that were burned, as in the concentrations of 1.5 and 1% (w/v) Virkon S.

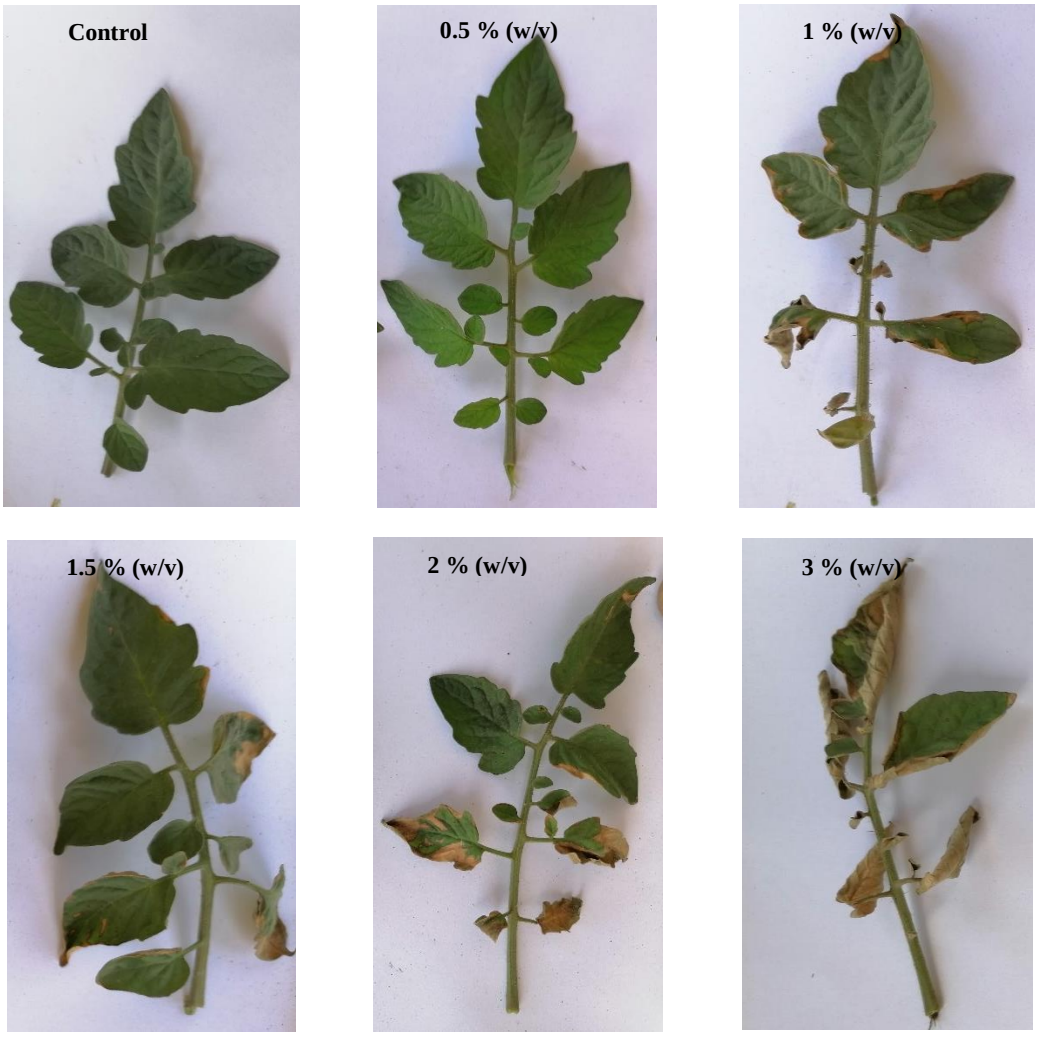

Figure 4: Phytotoxic effect of Virkon S after six days of treatment, tomato plant shoot system sprayed with serial concentrations of Virkon S applied with a hand-held sprayer.

Increasing the concentration of Virkon S to 3% (w/v) had a detrimental effect on the leaf tissues, resulting in damage to the majority of the tomato plant's leaf compound area

while preserving the life of some leaf tissues. The toxic effects of Virkon S can be regarded as local (non-systemic) toxic effects, as the leaves were the only plant components impacted, and the toxic effect of Virkon S begins at the leaf margins and moves within. Furthermore, the effect of Virkon S at low concentrations had no detrimental effect on the plant's blooms or newly produced green fruits, in contrast to the greater concentration of 3% (w/v), which nearly caused the death of the majority of the plant's developing top and flowers. The plant demonstrated a compensatory capacity and active bud formation in the developing apex's damaged region.

CONCLUSION

According to the present study's findings, the effectiveness of Virkon S as an effective nematode management tool has been proven against different stages of root-knot nematodes, whether egg masses, eggs, or the second juvenile stages, with high efficiency and a brief latency period. These observations confirm that Virkon S is an effective non-selective nematicide, whether on nematode developmental stages or species. When Virkon S was delivered by soil drenching, it worked well after 60 days enhancing tomato plant traits and causing oxidative stress with no observed phytotoxicity on tomato seedlings. Virkon S had a locally phytotoxic effect on tomato leaves after spraying the plant with concentrations greater than 0.5 % (w/v), causing leaf scorch injury, so the foliar application should be wised or avoided with higher concentrations. However, if Virkon S was not permitted to use it on nematode-infected plants, its effectiveness as a sterilization material for agricultural tools and machines, such as pruning shears, tractors, and harvesting machines, which are the primary transmission sources of nematode infestation, is a proven fact in the current study.

Finally, this makes Virkon S a potential and practical nematicide in the urgent need to reduce the number of nematodes in epidemic-infested soil after removing old vegetable crops and planting new crops. So, Virkon S could be applied at intervals between planting crops until further research is conducted to eliminate its limited use in plant production.

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الملخص العربي

النشاط الإبادي للفيركون إس ضد نيماتودا تعقد الجذور والإجهاد التأكسدي والسمية النباتية على الطماطم

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تحتاج الزراعة كصناعة عالمية البحث عن مواد كيماوية جديدة ذات خصائص فريدة للسيطرة على الآفات المستعصية مثل الأمراض القاطنة بالتربة. لذا اختبر مطهر الفيركون إس (Virkon® S) والمتكون من أوكسون (بيروكسي أحادي كبريتات البوتاسيوم) وسلفونات دوديسيل بنزين الصوديوم. وتهدف الدراسة لاستكشاف نشاط الفيركون كمبيد نيماتودى ضد نيماتودا تعقد الجذور *Meloidogyne incognita*، نظرا لنقص المعلومات المتاحة بهذا الصدد. عند تعريض كتل البيض لتركيز ٠,٤% (وزن\حجم) لمدة ساعة بأطباق بتري تجاوز معدل تثبيط فقس البيض ٥٠٪. علاوة على ذلك لوحظ قدرة الفيركون على إذابة المادة الجيلاتينية حول كتل البيض مسببا تشكل مجموعات صغيرة والتي سرعان ما تتحول لبيض حر مع انخفاض كبير في فقس البيض خلال مراحل التطور الجنيني المختلفة. بالإضافة إلى ذلك، أظهر الطور اليرقي الثاني فرط نشاط وإثارة غير عاديين قبل الإصابة بالشلل بعد أقل من ساعة من التعرض، ووصلت نسبة الموت لـ ٩٩,٢٠% بعد ١٢ ساعة تعرض لتركيز ١%. كما زادت سمية الفيركون بزيادة التركيز مصحوبا بقصر فترة الكمون خاصة ضد البيض. كما انعكس النشاط الفعال للفيركون ضد الأطوار اليرقية في التطبيق الناجح بعد تلقيح الطماطم بالأطوار اليرقية. حيث أظهرت مقاييس الوزن الغض للجذر والساق تحسنا واضحا بعد استخدام الفيركون، مع زيادة مئوية تناسبت طرديا مع التركيزات المستخدمة بعد ٦٠ يوما من تطبيقها. كما خفضت تركيزات الفيركون المختلفة بشكل كبير من الكثافة العددية لنيماتودا تعقد الجذور (البيض والطور اليرقي الثاني) كما خفضت أيضا من تكاثرها داخل التربة. علاوة على ذلك، نتج عن استخدام الفيركون بجميع تركيزاته انخفاض في أعداد العقد الجذرية وكتل البيض\جذر، في حين كان لتركيز ١٪ التأثير الأكبر في خفض معامل تكاثر النيماتودا. أدت الزيادة في تركيز الفيركون المستخدم غمراً للتربة إلى زيادة محتوى السكر الكلي والبروتين والفينول والفلافونويد، بالإضافة إلى انخفاض نشاط النترات والإنزيمات المؤكسدة في جذور الطماطم دون أي تأثير سام ملاحظ على نباتات الطماطم، إلا أن الرش على المجموع الخضري بتركيز أكبر من ٠,٥ ٪ (وزن / حجم) تسبب في حرق موضعي للأوراق. كما زادت شدة الأعراض عند تركيز ٣٪ (وزن \حجم)، مما أدى إلى موت الجزء العلوي من النبات وكما لوحظ نشاط البراعم الجانبية للنبات أسفل المنطقة المتضررة. وختاما، فقد أثبتت الدراسة فاعلية الفيركون كمبيد نيماتودي معتبر، ولكن هناك حاجة لمزيد من البحوث لرفع تقييد استخدمه في الإنتاج الزراعي.