Original Research Article

COMPATIBILITY OF VELUM® (SYNTHETIC NEMATICIDE) WITH ENDOPHYTIC *Colletotrichum nigrum* AND COMMERCIAL *Trichoderma asperellum* AGAINST ROOT-KNOT NEMATODES ON TREE TOMATO

ABSTRACT

Root-knot nematodes (RKNs)hinder agricultural production worldwide. Many methods of managingRKNs have been used. However, an approach of combiningvelum® with endophytic fungi andcommercial *Trichoderma asperellum* has not been tested against RKNs. This study was carried out between September 2022 to February 2023 to evaluated compatibility of Velum® with endophytic *Colletotrichum nigrum* isolated from roots of tree tomato and commercial *Trichoderma asperellum* to contain RKNs *in vitro* and in the greenhouse on tree tomato. Leadingmycelial discs from the colony of *C. nigrum*and*T.asperellum* were removed using a cork borer and inoculated onto PDA media amended with Velum® and incubated for seven days at 25^{o}C.*Colletotrichum nigrum* and *T. asperellum* alone and in combinations with Velum® were tested for their efficacy againstsecond stage juveniles (J2s) of RKN in the greenhouse. *In vitro* and greenhouse tests werereplicated four times in Completely Randomized Design. Data wasanalyzed by Anova SAS, 9.2 and significant means separated by Tukey's Honestly Significant Differences test at $P \leq 0.05$. There was nosignificant inhibition in the colony growth of *C.nigrum* and *T. asperellum*by Velum®*in vitro* and thereforewere found to be compatible.*Colletotrichum nigrum*combined with Velum® significantly ($P \leq 0.05$) reduced the RKN J2s, nematode reproduction factor, galling and egg mass indices in both experiments more than when applied alone. There was also a significant increase in plant growth parameters of tree tomato treated with *C. nigrum* combined with Velum®.This study has shown that combining Velum® with *C. nigrum*and *T. asperellum*successfully controlled RKNs on tree tomato. These findings could be used to promote integrated nematode management using less toxic nematicides and biocontrol agents.

Key words: Root-knot nematodes; second stage juveniles (J2s), *Colletotrichum nigrum*; Velum®; *Trichoderma asperellum*, Endophytic fungi

1. INTRODUCTION

Tree tomato (*Solanum betaceum* Cav.) belongs to family Solanaceae, a perennial crop that is grown in tropical and subtropical climatic conditions (Ramírez-Gil, 2017). In Kenya, it is an economically important horticultural crop that contributes to rural income generation (Muriithi *et al*., 2013)and has high nutritional value (AFA, 2021) as well as contributingover 8 million US dollars annually. The fruits are oval with thick, smooth and shiny covering. When ripe, the fruits are consumed raw or turned into juice. Iron, phosphorus, potassium, calcium, antioxidants, and vitamins A, B, C, and E are abundant in the fruits (Pedrosa, 2016). These nutritional benefits make it to be popularly cultivated around the world.

However, the roots of this crop are seriously attacked by root-knot nematodes (RKNs) causing losses and lowering the yields (Waswa *et al*., 2020). Root-knot nematodes are a major threat to horticultural crops across the world. TheRKNs cause serious damage to roots and severely reduce yields of many crops such as tree tomatoes, tomatoes, bananas (Jones *et al*.,

2013). Root-knot nematodes are widely distributed in most agricultural soils attacking over 3000 plants globally and causing more than 100 billion US dollarsas losses each year threatening development of sustainable agriculture (Huang *et al*., 2006; Jones *et al*., 2013). Infestation with root-knot nematode leads to stunted growth, chlorosis and root shortening. They also cause root galls which interfere with the plants' ability to absorb water and mineral nutrients. The RKNs also cause above-ground symptoms that are usually confused with mineral deficiencies and other diseases (Dahlin *et al*., 2019).

Managing RKNs is difficult because of their high rate of multiplication and short life cycle (Trudgil and Blok, 2001). Several methods have been used to control RKNs. Previously; RKNs have been controlled significantly by broad spectrum chemicals that are harmful to users and the environment. Therefore, alternative methods of management are required to reduce the usage of harmful chemicals (Huang *et al*., 2018). With the increasing demand to meet the challenges of food and nutritional security, management of RKNs is no longer a single approach but integrative strategies (D'errico *et al*., 2019). Going forward, the control of RKNs should not only focus on sustainable methods of management but also by wise use of chemical nematicides for holistic management (Chen *et al*., 2020; Orlando *et al*., 2020). Potential biocontrol agents like endophytic fungi that are antagonistic to RKNs are important. Endophytic fungi have shown efficacy against nematodes through production of toxic secondary metabolites, sticky hyphae and colonization of nematode eggs and bodies (Li *et al*., 2015). Endophytic fungi present a significant strategy of managing RKNs in promoting sustainable agriculture. However, there has been no single method that has been able to eradicate RKNs and biological control agents (BCAS) e.g. *Purpureocillium lilacinum* have shown inconsistencies in their performance against RKNs in the greenhouse and in the field (Gine and Sorribas, 2017). Their potential in the management of RKNs can be improved by combining with nematicides which have low toxicity profiles such as Velum® (active ingredient =fluopyram) to leverage on their synergistic effects (Dahlin *et al*., 2019). Application of endophytic fungi in combination with new chemical nematicides could be a more attractive approach than applying each one of them alone or singly (Dahlin *et al*., 2019). Velum® has been effectively used against RKNs (Singh *et al*., 2017; Dahlin *et al*., 2019). In consideration, non-chemical management of RKNs is a cost effective approachalthoughit is sometimes less effective than chemical nematicides. Thus, use of non-chemical approaches in combination with compatible chemicals could be used in comprehensive management of RKNs (Chen *et al*., 2020).However, there has been no documentation on the efficacy of combining endophytic *Colletotrichum nigrum* from roots of tree tomato with Velum® against RKNs. Thus, using lower ecotoxicologically new chemicals such as Velum®in combination with endophytic fungi is currently preferred (Chen *etal*., 2020; Dahlin *etal*., 2019).Therefore, combining such chemicals with endophytic *C. nigrum* to manage RKNs might be a better strategy than applying each of them individually.Thakur *et al*. (2020) found that integrating metham sodium with neem cake and *Purpureocillium* reduced nematode populations in rhizosphere soil and roots of cucumber. Kumar *et al*.(2017) also found that combining organic amendments, *Trichoderma* spp. and carbofuran significantly reduced rice root-knot nematodes, *M. graminicola* and also increased growth and yields of rice higher than when used as single treatments. There has also been no documented studyhighlighting the effects of Velum® on the performance of endophytic *C. nigrum* and *T. asperellum*against RKNs on tree tomato and hence the importance of this study.

The main objective of this study was to evaluate the compatibility of chemical nematicide Velum® with endophytic *C. nigrum*and *T. asperellum*to demonstrate their synergistic effect in

the management of RKNs*in vitro* and in the greenhouse. In this study, Velum® combined with endophytic *C. nigrum* and *T. asperellum* significantly reduced RKN populations and increased plant growth parameters of tree tomato.

2. METHOLODOLGY

2.1 Sampling

Tree tomato (*Solanum betaceum*Cav.) root samples were collected from thirty farms randomly selected using purposive sampling technique (Suri, 2011). Ten fresh healthy rootswere collected from each farm in Kinangop sub-county (-.5546 ^{o} S, 36.5536 ^{o} E) located in Nyandarua County lying at 0.1804^{o}S and 36.5230^{o}E in Kenya. The roots were put into zip lock bags and transported to Kenyatta University Agriculture Laboratory for processing. The *in vitro* and greenhouse experiments were carried out at Kenyatta University main campus (1.1805^{o} S and 369348^{o} E).

2.2 Isolation of endophytic fungi from tree tomato roots

Sterilization of roots was done according to Dababat *et al*. (2008) protocol. The roots were cut into 5 cm length, thoroughly washed with tap water and sterilized with 70 % ethanol for 3 minutes to remove surface epiphytes. The roots were then sterilized in 1.5 % Sodium Hypochlorite (NaOCl) for three minutes. Sterilized roots were rinsed three times with sterile distilled water; blot dried using sterile blotting papers and cut into 0.5 cm length using sterile scalpel blades (Dababat *et al*., 2008). The 0.5 cm root pieces were evenly placed on sterilized potato dextrose agar (PDA) media amended with 150 mg/l each of streptomycin-sulphate to inhibit bacteria contamination. The PDA plates were sealed with parafilm and incubated at 25 ^{o}C until endophytic fungi emerged. To evaluate the integrity of sterilization, water from the last rinse was plated onto a fresh PDA media and incubated at 25 ^{o}C for seven days. To obtain pure cultures, fungal cultures of the isolate were sub-cultured on a fresh PDA media using discs from the leading mycelia margins taken by flame sterilized 5 mm cork borer and incubated for two weeks.

The endophytic fungus was identified morphologically using macroscopic characteristics of the colonies (colour, margin, texture, and density - Plate 1: A, B) and microscopic features of conidia and mycelia under the microscope stained by Lactophenol cotton blue (Plate 1: C).

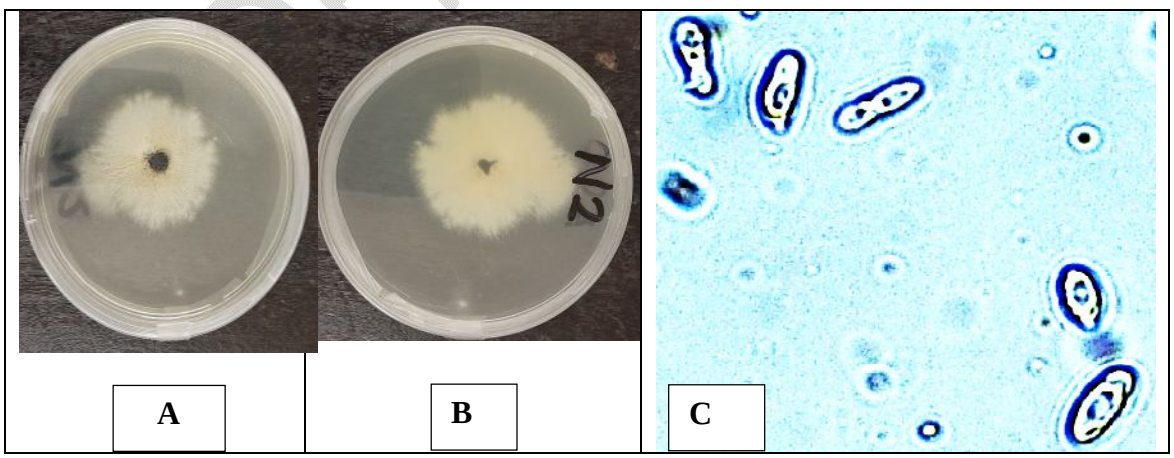

Plate 1: A:*C. nigrum* colony Reverse, **B:** *C. nigrum* colony Front and **C:***C. nigrum* conidia**(×1000**)

2.3*In vitro*test

Onemilliliter of Velum® suspension was dispensed into a 1 L conical flask containing 1L sterilized molten PDA media and gently agitated for even mixing. The media was amended with 150 mg/l each of streptomycin-sulphate.The mixture was then poured into 90 mm petri dishes and allowed to cool and solidify. The discs of mycelia from actively growing margin of the colonies of fungi were removed using sterile 5 mm cork borers and inoculated onto fresh PDA media amended with Velum®. Control plates contained un-amended PDA media inoculated with either of the fungus. The PDA plates were sealed with parafilm and incubated for seven days at 25 degrees Celsius. Commercial *Trichoderma asperellum* was used as a check for comparison with *Colletotrichum nigrum* isolate. The treatments were replicated fourtimes each and arranged in completely randomized design (CRD). The treatments included:

i) Plates amended with Velum®+ endophytic *Colletotrichum nigrum*
ii) Plates amended with Velum®+ Commercial *Trichoderma asperellum*
iii) Un-amended Plates treated with *C. nigrum*
iv) Un-amended Plates treated with *T.asperellum*

The colony diameter from radial growth was measured from the underside of each petri dish. The percentage inhibition of growth over the control was calculated using the formula below:

$$\mathbf{I} = \frac{\mathbf{C}-\mathbf{T}}{\mathbf{C}} \times \mathbf{100}$$ (Behdani *et al.*, 2012)

Where **I** = percent inhibition, **C** = colony diameter in control plate, and **T** = colony diameter in treated plate. The experiment was repeated once for accuracy.

2.4 Greenhouse experiment

2.4.1 Mass multiplication of endophytic fungi

The spores of the fungi were mass-multiplied using sorghum grains following the procedures of Cumagun and Moosavi. (2015). The grains were soaked in water, sterilized in an autoclave and inoculated with four 5 mm discs of 5 day old cultures per 200g and incubated in black polythene bags for 14 days. The grains were dried, blended into powder and passed through 50µm sieve. The spore powder was then mixed thoroughly with sterile talc powder (carrier material) in 1:2 ratio and Carboxymethyl cellulose (sticking agent) at 5 g/kg of the product (Sing *et al*., 2016). Accurate spore density (1×10^6 spores/ml) of the fungi was determined by a hemocytometer under the microscope.

2.4.2 Preparation of growth media for greenhouse experiments

A mixture of 2 sand to 1 soil proportion was thoroughly mixed and heat-sterilized to 100 degrees Celsius for 48 hours. To prevent contamination, 2 kilograms of the sterilized media were placed in thoroughly cleaned plastic pots (12-cm-diameter) that had been sterilized with 1.5% of NaOCl.

2.4.3 Nursery establishment

A certified nursery (Kenya Agriculture, Livestock and Research Organization at Horticulture Research Institute) provided the susceptible cultivar of tree tomato seeds, which were then immersed in sterilized distilled water for a day. After soaking, the seeds were placed in sterile petri plates, lined with moist tissue paper and wrapped with Parafilm. The petri plates were covered with aluminum foil and kept on the benches in the greenhouse for 12 to 15 days. Following germination, the seeds were placed in sterile media-filled germination trays and maintained for four weeks before being transplanted into pots.

2.4.4 Preparation of nematode inoculum

Root-knot nematode J2s were reared on a susceptible tomato variety (Cal-J) for three months in the Agriculture greenhouse at Kenyatta University. Galled roots of tree tomato were used to obtain nematode inoculum. An egg mass from a single female was picked using a needle to establish pure cultures for the experiments. The RKN egg mass was put at the root zone of four week old transplanted susceptible tomato plants of cultivar Cal- J. in pots with sterile sand-soil mixture media and maintained in the greenhouse. After three months, plants were uprooted, galled roots were washed, chopped into 1cm, macerated in 1.5 % NaOCl solution in a blender and the suspension passed through 500 µm, 106 mµ and 20 µm sieves into a beaker (Hooper et al., 2005. The resultant suspension containing RKN eggs was incubated on plates lined with a serviette in darkness for 14 days and freshly hatched J2s were collected from fourth day every two days (Coyne *et al*., 2007). The numbers of freshly hatched J2s per ml were determined under the microscope at ×40 using grid-49 nematode counting dish (Hussey and Barker, 1973). The nematode suspension was adjusted with sterile distilled water to 2000 J2s/ml for use per pot in the greenhouse.

2.4.5Greenhouse treatments

Sterile media was placed into sterile plastic pots in the greenhouse. At transplanting, 40 ml of 1 ml/L Velum® nematicide was applied to the soil in pots. One four-week old tree tomato seedling was then transplanted per pot. Seedlings were treated with 40 ml of 1×10^6spores/ml of either endophytic *Colletotrichum nigrum* or commercial *Trichoderma asperellum* as soil drench at transplanting. Pots were inoculated with 2000 J2s of RKN each and herein referred to as inoculated. Pots treated with treatments other than J2s are herein referred to as treated. Pots inoculated with J2s of RKN only served as positive control while un-treated pots without RKN J2s served as negative control (absolute control). The treatments had four replicates each and arranged in CRD. The treatments were as follows:

i) *Colletotrichum nigrum* + Velum® + J2s
ii) *C. nigrum* + Velum® - J2s
iii) *Trichoderma asperellum* + Velum® + J2s
iv) *T. asperellum* + Velum® - J2s
v) *C. nigrum* + J2s
vi) *C. nigrum* - J2s
vii) *T. asperellum* + J2s
viii) *T. asperellum* - J2s
ix) Velum® + J2s
x) Velum® - J2s
xi) J2s alone
xii) Untreated and un-inoculated

The experiments were terminated 90 days after transplanting.

2.4.6Evaluation of root-knot nematode disease parameters

At the end of 90 days, the J2s of RKN were extracted from 200 cm^3 of rhizosphere soil or 5 g of roots using modified Baermann technique (Hooper *et al*., 2005; Coyne *et al*., 2007). Five grams of roots were washed and chopped into 1 cm pieces and macerated in a blender for one minute. Nematodes were extracted using the above technique. The set-ups were incubated for 48 hours for nematode recovery. Then the nematode suspension was poured into 20µm sieve, backwashed gently and collected into a beaker and concentrated to 10 ml using a test sieve. Then 2 ml suspension of nematodes was pipetted into a nematode counting dish and nematodes

counted at ×10 under dissecting microscope and enumerated from the original concentrated suspension for each sample. The process was repeated thrice for each sample to get the average. The nematodes were expressed per 200 cm^3 of dry soil or 5 g of roots.

Galling and egg mass indices were determined using a scale of 0-5 (Quesenberry *et al*., 1989) as shown in Table 1. Individual root systems were washed, and galling/egg mass indices were scored.

Table 1: Scoring scale for root-knot nematode galling and egg mass indices

Score	Description
0	No galls or No egg masses per root system
1	1-2 galls or egg masses per root system
2	3-10 galls or egg masses per root system
3	11-30 galls or egg masses per root system
4	31-100 galls or egg masses per root system
5	>100 galls or egg masses per root system

Phloxine-B (Holbrook *et al*., 1983) was used to stain root systems to visualize egg masses and RKN females. The roots were immersed in phloxine-B for 20 minutes (Daykin and Hussey, 1985), and then rinsed in tap water to remove excess stain before viewing under a dissecting microscope.

The nematode reproduction factor (Rf) was determined from final nematode populations (Pf) and initial nematode populations (Pi) expressed as a ratio of Pf: Pi.

2.4.7 Evaluation of plant growth parameters

A centimeter graduated ruler was used to measure plant shoot height from the soil baseline to the newest apical shoots at the conclusion of the experiment.

At the end of the experiment, the dry weights of root and shoot were measured in grams. Plants were gently uprooted, and shoots and roots were separated by cutting at the base. To remove adhering soil, the roots were washed in flowing water under the tap and dried with blotting paper. The shoots and roots were placed in separate paper bags with clear labels and oven-dried at 60^{o}C for three days to achieve constant mass for measurement of dry weights of shoot and root.

2.5 Data analysis

The data was organized in excel sheets and analyzed using SAS version 9.2 computer software's one way analysis of variance (ANOVA). Tukey's Honest Significant Differences (HSD where $P \leq 0.05$) was used to separate the significant means. Regression and correlation analysis were done to evaluate the relationship between RKN disease parameters

3. RESULTS

3.1 Compatibility of Velum® with endophytic *Colletotrichum nigrum*and *Trichoderma asperellum* against J2s of RKN

3.1.1 Effect of Velum® on colony growth of endophytic *Colletotrichumnigrum* and *Trichoderma asperellumin vitro*

There was no significant difference ($P \geq 0.05$) in the colony diameter of endophytic *C. nigrum* exposed to Velum® compared to the untreated control (*C. nigrum* alone) as shown in Table 2 (experiments I and II) and plate 2. Commercial *T. asperellum*colony diameter did not

also differ significantly with the un-treated control (*T. asperellum* only) on exposure to Velum® in experiment I and II as shown in Table 2 and plate 2.

Table 2: Effect of Velum® on growth (colony diameter) of *Colletotricum nigrum* isolate and *Trichoderma asperellum*

	Expt. I	**Expt. II**
Treatments	**% Inhibition**	**% Inhibition**
C. nigrum + Velum®	1.00 ± 0.79a	1.02 ± 0.73a
C. nigrum alone (control)	0.00 ± 0.00a	0.00 ± 0.00a
T. asperellum+ Velum®	1.01 ± 0.58a	0.99 ± 0.33a
T. asperellum alone (control)	0.00 ± 0.00a	0.00 ± 0.00a
P-value	0.29	0.27

Data are means ± SE of four replicates. Means followed by the same letter(s) on the same column are not significantly different (P≥0.05) according to Tukey's Honestly Significant Difference (HSD) test

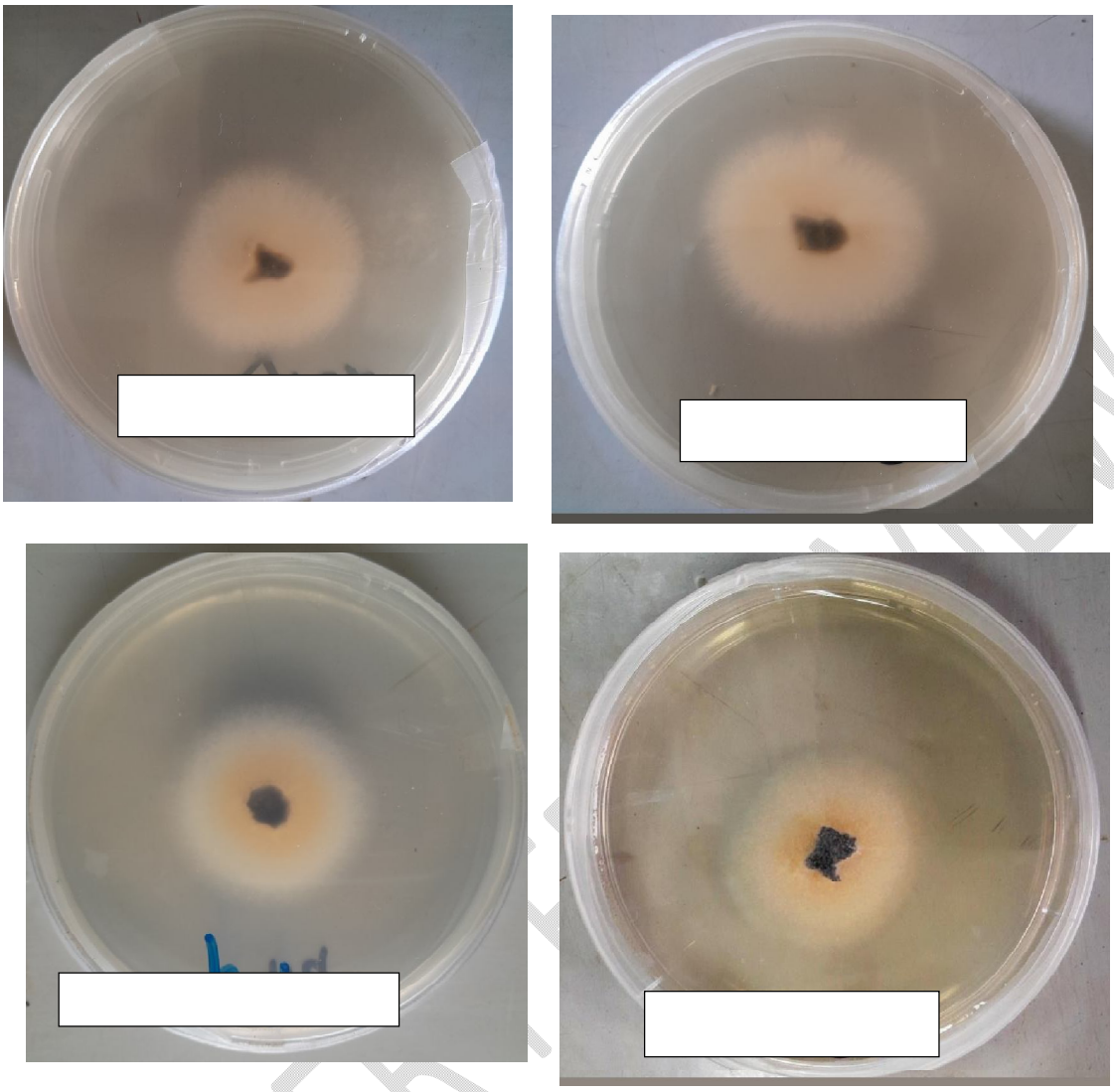

Plate 2: Effect of Velum® on colony diameter of endophytic *Colletotricum nigrum* and commercial *Trichoderma asperellum in vitro*

3.1.2 Efficacy of combining Velum® with *Colletotrichum nigrum* and *Trichoderma asperellum* against RKNs on tree tomato; Greenhouse tests I and II

3.1.2.1 Effect on second stage juveniles of RKN population in soil and roots

The soil population of J2s differed significantly ($P \leq 0.05$) among the treatments (Table 3) Nematode-inoculated plants treated with *C. nigrum* + Velum® had the lowest population of J2s per 200 cc of dry soil followed by those treated with *T. asperellum* + Velum® and Velum® alone, respectively (Table 3). All treatments significantly reduced nematode populations in the rhizospheric soil compared to the positive control.

The second stage juvenile populations (J2s) in roots were statistically different ($P \leq 0.05$) between all treatments. Nematode-inoculated plants with *C. nigrum* + Velum® treatment had the lowest J2s per 5g of dry roots followed by those treated with *T. asperellum* + Velum® and Velum® alone, respectively (Tables 3). All treatments significantly reduced RKN J2 populations in roots as compared to the positive control.

3.1.2.2 Effect on RKN reproduction factor

The RKN reproduction factor was statistically different ($P \leq 0.05$) between different treatments (Table 3). The *C. nigrum* + Velum® treatment significantly reduced RKN reproduction factor followed by *T. asperellum* + Velum® and Velum® alone relative to positive control.

Table 3: Efficacy of combining Velum® with *Colletotrichum nigrum* and *Trichoderma asperellum* on J2 populations and RKN reproduction factor on tree tomato in the greenhouse tests I and II

	RKN disease parameters					
	Greenhouse test I			Greenhouse test II		
Treatments	**J2s in soil**	**J2s in roots**	**RF**	**J2s in soil**	**J2s in roots**	**RF**
C.nigrum + J2s	549.17 ± 24.85b	186.25 ± 23.04b	0.28 ± 0.01b	542.50 ± 12.96b	182.50 ± 31.52b	0.27 ± 0.01b
T. asperellum + J2s	580.83 ± 18.00b	198.75 ± 12.14b	0.29 ± 0.01b	574.17 ± 31.56b	191.25 ± 4.73b	0.29 ± 0.02b
C. nigrum + Velum® + J2s	215.83 ± 9.26d	45.00 ± 28.50d	0.11 ± 0.01cd	208.33 ± 12.29d	41.25 ± 4.27d	0.10 ± 0.01cd
T. asperellum + Velum® + J2s	226.67 ± 5.27d	57.50 ± 12.50d	0.14 ± 0.02cd	219.17 ± 9.44d	51.23 ± 2.39d	0.11 ± 0.01cd
Velum® + J2s	303.27 ± 6.21c	111.35 ± 9.05c	0.17± 0.03c	312.00 ± 14.07c	107.25 ± 9.08c	0.15 ± 0.02c
Positive control (J2s alone)	3223.33 ± 30.46a	516.25 ± 30.44a	1.61 ± 0.02a	3211.67 ± 113.81a	498.75 ± 14.63a	1.61 ± 0.06a
Negative control	0.00 ± 0.00e	0.00 ± 0.00e	0.00 ± 0.00d	0.00 ± 0.00e	0.00 ± 0.00e	0.00 ± 0.00d
P-value	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001

Data are means ± SE of four replicates. Means followed by different letter(s) in the same column are significantly different according to Tukey's Honestly significant difference (HSD) test at P≤0.05. **J2s** = second stage juveniles of RKNs; **RF** = Nematode reproduction factor calculated as a ratio of final nematode populations (Pf) to initial nematode populations (Pi). Negative control = untreated control without nematodes.

Regression analysis indicated a positive linear association between nematode reproduction factor (Rf) and RKN J2 populations (Figure 1). A further investigation showed that the Rf and soil J2 populations correlated positively (r=1, P=0.05).

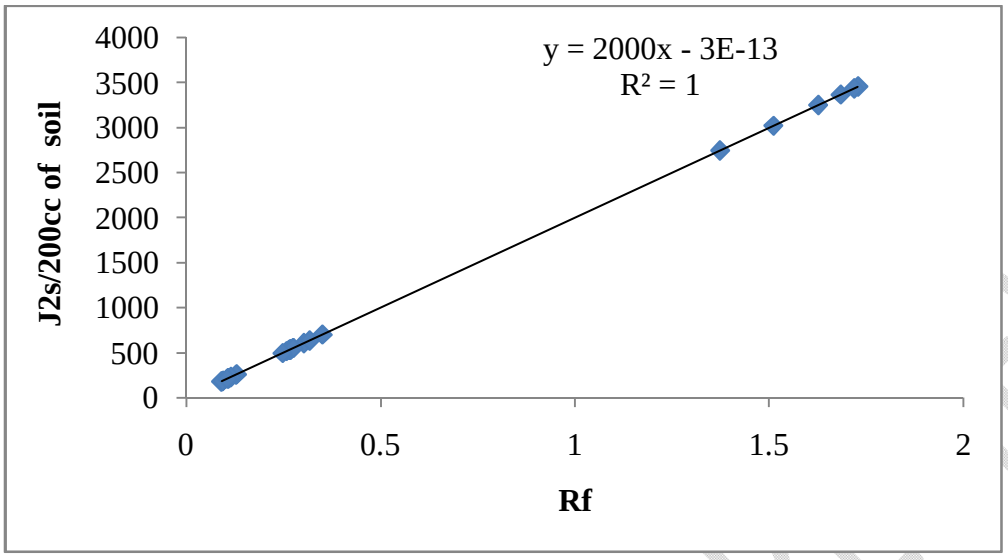

Figure 1: Relationship between nematode reproduction factor and J2 populations in the soil (Greenhouse test)

3.1.2.3 Effect on galling index and egg mass index

There was an existence of statistical difference (P≤0.05) in terms of indices on gall and egg masses among the treatments (Table 4). Plants treated with *C. nigrum* + Velum® had the lowest gall and egg mass indices followed by those treated with *T. asperellum* + Velum® and Velum® alone as shown in Table 4. The least gall and egg mass indices reduction was shown in *T.asperellum* standalone treatment.

Table 4: Efficacy of combining Velum® with *Colletotrichum nigrum* and *Trichoderma asperellum* on indices of galling and egg masses on tree tomato in the greenhouse tests I and II

	Plant disease parameters			
	Greenhouse test I		Greenhouse test II	
Treatments	**GI**	**EMI**	**GI**	**EMI**
Colletotrichum nigrum + J2s	3.67 ± 0.21b	2.75 ± 0.25bc	3.83 ± 0.09b	3.00 ± 0.00b
Trichoderma asperellum + J2s	3.83 ± 0.17b	3.25 ± 0.25b	3.67 ± 0.56b	3.33 ± 0.50b
C. nigrum + Velum® + J2s	1.50 ± 0.43cd	1.50 ± 0.29cd	1.00 ± 0.45d	1.17 ± 0.31d
T. asperellum + Velum® + J2s	2.00 ± 0.43cd	2.00 ± 0.00c	1.50 ± 0.43d	1.33 ± 0.33d
Velum® + J2s	2.25± 0.51 c	2.05± 0.01c	2.00 ± 0.50c	2.00 ± 0.00c
Positive control (J2s alone)	5.00 ± 0.00a	5.00 ± 0.00a	5.00 ± 0.00a	5.00 ± 0.00a
Negative control (untreated/uninoculated)	0.00 ± 0.00d	0.00 ± 0.00d	0.00 ± 0.00e	0.00 ± 0.00e
P-value	<.0001	<.0001	<.0001	<.0001

Data are means ± SE of four replicates. Means followed by different letter(s) in the same column are significantly different according to Tukey's Honestly significant difference (HSD) test at P≤0.05. **J2s** = second stage juveniles of RKNs;**GI** = Galling index; **EMI** = Egg mass index; Negative control = untreated control without nematodes. GI and EMI were scored on a scale of 0-5, where 0 = no gall/egg masses; 1=1-2 galls/egg masses; 2=3-10 galls/egg masses; 3=11-30 galls/egg masses; 4=31-100 galls/egg masses and 5=>100 galls/egg masses (Quesenberry *et al*., 1989).

3.1.2.4 Effect on shoot height

The heights of plants in various treatments significantly differed ($P \leq 0.05$) from one another. The heights of uninoculated (without nematodes) plants treated with *C. nigrum* and *Trichoderma asperellum* as standalone treatments had statistically higher ($P \leq 0.05$) shoots than those of the negative control (without nematodes and without treatments) as shown in Table 5. The heights of nematode-inoculated *C. nigrum* + Velum®, *T. asperellum* + Velum®, *C. nigrum* and *T. asperellum* treated plants were significantly higher than those of the positive control (nematodes alone). The results showed that all treatments increased the height of shoots of tree tomato relative to the positive control (Plate 3 and Table 5).

Plate 3: Effect of combining *Colletotrichum nigrum* and *Trichoderma asperellum* with Velum on shoot height of tree tomato in the greenhouse test.

a - h represents treatments where; **a** = innoclated + *Colletotrichum nigrum*; **b** = innoclated + *Trichoderma asperellum*; **c** = innoclated + *C. nigrum* + Velum®; **d** = innoclated + *T. asperellum* + Velum®; **e** = negative control (un-treated and un-innoculated); **f** = Positive control (un-treated and inoculated); **g** = *C. nigrum* alone; and **h** = *T. asperellum* alone

3.1.2.5 Effect on dry weights of root and shoot

There was significant difference (P≤0.05) in dry root weight of plants among different treatments (Table 5). The highest dry root weight was recorded in un-inoculated (without nematodes) *C. nigrum* treated plants followed by un-inoculated plants treated with *T. asperellum* and negative control (untreated and un-inoculated).

The dry shoot weights were significantly different ($P \leq 0.05$) among the treatments (Table 5). The dry shoot weights of un-inoculated and treated plants were significantly higher than those of the inoculated and the positive control. Un-inoculated plants treated with *C. nigrum* and Un-inoculated plants treated with *T. asperellum* had higher dry shoot weights as compared to the negative control (Table 5).

Table 5: Effect of combining Velum® with *Colletotrichum nigrum* and *Trichoderma asperellum* on shoot height (cm) and dry weights (gms) of tree tomato in the greenhouse tests I and II

	Plant growth parameters					
	Greenhouse test I			**Greenhouse test II**		
Treatments	**SH**	**DRW**	**DSW**	**SH**	**DRW**	**DSW**
C. nigrum + Velum® + J2s	35.18 ± 0.64c	4.56 ± 0.07b	8.25 ± 1.5b	35.67 ± 2.57d	4.20 ± 0.40b	9.19 ± 1.32b
C. nigrum + Velum® - J2s	43.98 ± 1.49ab	6.84 ±0.65 ab	10.25 ± 0.25ab	45.87 ± 1.11b	6.27 ± 0.58ab	11.34 ± 0.50a
T.asperellum + Velum® + J2s	34.37 ± 1.76c	3.99 ± 0.79c	8.08 ± 0.92b	35.05 ± 2.00d	3.85 ± 0.21b	9.01 ± 1.14b
T. asperellum + Velum® - J2s	43.81 ± 1.44ab	6.72 ± 0.69b	10.13 ± 0.96ab	45.03 ± 1.10b	6.23 ± 0.50ab	11.17 ± 0.53a
C. nigrum + J2s	33.22 ± 1.77c	3.11 ± 0.12c	7.87 ± 0.31c	34.92 ± 2.25d	3.09 ± 0.34bc	8.71 ± 1.06b
C. nigrum - J2s	46.87 ± 1.41a	7.64 ± 0.16a	11.65 ± 0.43a	48.67 ± 0.77a	7.15 ± 0.26a	12.14 ± 0.38a
T. asperellum + J2s	33.02 ± 0.59c	3.00 ± 0.66c	7.10 ± 0.53c	33.91 ± 1.15d	2.93 ± 0.24bc	8.64 ± 0.51b
T. asperellum - J2s	46.53 ± 0.50a	7.31 ± 0.48a	11.22 ± 0.27a	48.33 ± 0.98a	7.05 ± 0.39a	11.80 ± 0.56a
Velum® + J2s	34.01 ± 1.70c	4.14 ± 0.88b	6.99 ± 1.45c	34.47 ± 2.21d	4.03 ± 0.38b	6.09 ± 1.12c
Velum® - J2s	39.10 ± .0 57b	4.85 ± 0.81b	7.03 ± 0.41c	40.21 ± 0.99c	4.22 ± 0.40b	7.06 ± 1.09c
Positive control (J2s alone)	26.08 ± 0.64d	2.03 ± 0.33d	5.09 ± 0.20d	29.25 ± 1.91e	1.97 ± 0.20c	5.84 ± 0.43d
Negative control	42.63 ± 1.52ab	6.37 ± 0.64ab	10.02 ± 0.26ab	44.50 ± 1.68b	6.15 ± 0.49ab	10.47 ± 0.44ab
P-value	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001

Data are means ± SE of four replicates. Means followed by different letter(s) in the same column are significantly different according to Tukey's Honestly significant difference (HSD) test at P≤0.05. **J2s** = second stage juveniles of RKNs;**SH** = shoot height; **DRW** = Dry root weight and **DSW** = Dry shoot weight. Negative control = untreated control without nematodes.

4. DISCUSSION

4.1 Compatibility of Velum prime with endophytic *Colletotrichumnigrum* and commercial *Trichoderma asperellum* against RKNs on tree tomato

Velum® did not inhibit the colony growths of *C. nigrum* and *T. asperellum* on PDA media. These results corroborate a research carried out by Kibunja. (2015) on the effect of selected endophytic fungi and resistant tomato cultivars on *Meloidogyne* spp. The author tested the compatibility of Mocap (active ingredient: ethoprophos= organophosphate) and *Trichoderma* spp. and reported that Mocap had no significant effect on *Trichoderma* isolates. In this study, this could be due to Velum® being a systemic non-fumigant is relatively less toxic to soil microflora (Wada and Toyota, 2008). Non-fumigants are said to have little effect on organisms that lack the nervous system since these nematicides act as cholinesterase inhibitors in the neurons (Bhattacharjee and Dey 2014).

The reduction in plant disease parameters could be due to *C. nigrum* and *T. asperellum* producing nematotoxic secondary metabolites (flavonoids, terpenoids, trichodermin, colletonoic acid etc) against RKN J2s or directlyparasitizing them. Other studies have shown that biocontrol endophytic fungi produce toxic metabolites against RKNs (Kumar *et al*., 2023; Yao *et al*., 2023; Meyer *et* al., 2020). Velum® is known to act against soil pathogens and enhance plant health (Chen *et al*., 2020). The nematicidal effect of Velum® on nematodes has been variously described (Beeman and Tylka, 2018; Faske and Hurd, 2015; 2016; Roper, 2017). This could explain the reduced disease parameters by the combined application of biocontrol fungi with Velum®. Other studies have highlighted the nematicidal effect of Velum® (Dahlin, *et al*., 2019). *Colletotrichum nigrum* could have worked synergistically with Velum® against the RKN J2s resulting into decreased disease parameters and enhanced plant growth. These results are in agreement with Dahlin, *et al*. (2019) who noted that combining *Purpureicillium lilacinum* (strain PL251) with Velum® reduced J2 populations of *M. incognita* by 68% and gall index to 1.8 as compared to control (3.8). The same treatment also enhanced yield of tomatoes under greenhouse tests. Muthulakshmi*et al*. (2012) also found that combining carbofuran with biocontrol agents significantly increased the number and weight of potato tubers per plant and reduced potato cyst nematode populations in potatoes.

The biocontrol fungi also increased the plant growth parameters of tree tomato when applied alone and in combination with Velum® in the greenhouse tests. As standalone applications, these biocontrol fungi (*C. nigrum* and *T. asperellum*) could be possessing plant growth promoting abilities. This could explain the increased shoot heights and dry weights of root and shoot of tree tomato plants. Some fungi such as*Trichoderma* spp. are known to act as biofertilizers which improves plant growth (Kubheka and Ziena, 2022). *Trichoderma* spp. have been used to produce volatile chemicals (alkaloids, colletonoic acid, esters, alcohols, ketones e.t.c) against soil pathogens, solubilizes phosphates to make them available for plant absorption in acidic soils and also enhances uptake of micro and macro- nutrients by plants (Kubheka and Ziena, 2022). The endophytic fungi are also known to confer protection to plants against other pathogens (Silva Santos *et al*., 2022) and this could explain the reduced plant disease parameters shown by their application in this study.Another research showed that endophytic *Colletotrichum tofieldiae* promoted plant growth of maize and tomato *in-vitro* resulting into higher shoot heights and weights (Conzalez-Diaz Sandra, *et al*,. 2020). Silva Santos *et al*. (2022) also found out that inoculating tomato plants with *Colletotrichumsiamense* increased plant biomass. As combined treatments (Biocontrol fungi + Velum® were able to enhance plant growth parameters (shoot

height and dry weights) and also reduced plant disease parameters (nematode populations, egg masses and gall indices and reproduction factor).

Tree tomato farmers should utilize cost effective RKN management options to maximize on their profit. This may involve use of biological control agents (BCAs) and/or nematicides. This could consist of combining compatible nematicides with biological agents for their additive effects against RKNs. The findings of this study could be used to promote integrated nematode management using less toxic nematicides and biocontrol agents especially under field conditions where efficacy of BCAs is limited (Köhl*et al*, 2011).

5. CONCLUSION

Velum® was found compatible with endophytic *Colletotrichum nigrum* and commercial*T. asperellum*. This study demonstrated that their co-application significantly reduced root-knot nematode populations in the soil and roots of tree tomato and therefore have potential of being used in integrated nematode management especially under field conditions where efficacy of BCAs is erratic. The integration of chemical nematcides and biological approaches may become a critical component in the management of RKNs. Integrated nematode management (INM) may not only be used for different categories of compatible RKN management but also embrace utilization of different components of BCAs in combination with favourable chemicals that have low ecotoxicity profiles.

6. RECOMMENDATION

Further research should be conducted to determine the mechanisms of action of the endophytic*Colettotrichumnigrum*on RKN J2s. Apart from Velum®, other combinations should be tested for compatibility and applied as integrated approach rather than as standalone in the management of RKNs.

Data availability

All data supporting the findings of this study are presented in this paper.

Disclaimer (Artificial intelligence)

Option 1:

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

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