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Suppression of *Meloidogyne javanica* Infection in Peach (*Prunus persica* (L.) Batsch) Using Fungal Biocontrol Agents

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Abstract: In South Asian countries, the lucrative production of peaches has been seriously threatened by an assortment of biotic stresses especially nematodes. This situation compromises the achievement of sustainable development goals (SDGs) related to food security and zero hunger. Recently under changing climate, root-knot nematodes of the genus *Meloidogyne* have emerged as the most damaging phytopathogenic nematodes, while the efficacy of chemical control has remained limited. Therefore, a study was executed to assess the efficacy of four biocontrol agents including *Pochonia chlamydosporia*, *Purpureocillum lilacinum*, *Trichoderma harzianum*, and *T. viride* (at concentrations of 2.5×10^3, 5×10^3, 7.5×10^3, and 1×10^4) along with nematicide Rugby and a control treatment against *Meloidogyne javanica* on peach. The response variables included nematode infestations in terms of number of galls, egg masses, and reproductive factors. *P. lilacinus* and *T. harzianum* (1×10^4 concentration) reduced the number of galls by 18% and 16%, respectively, than the control. All biocontrol agents exhibited their effectiveness by significantly reducing number of egg masses, eggs per egg mass, and reproductive factors, while these remained statistically at par to each other. The study proved that application of these biocontrol agents holds potential for controlling root-knot nematodes and might be developed as a potent strategy to replace or at least reduce the use of traditional chemicals for avoiding environmental pollution and contamination.

Keywords: ecofriendly management; reproductive factor; prunus persica; egg masses

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1. Introduction

Globally, the sustainable development goals (SDGs) especially poverty alleviation and zero hunger are directly linked with the sustainable productivity and profitability of agricultural produce. Among stone fruits, peach (*Prunus persica* (L.) Batsch) occupies a central position as it is grown in all habitable continents of the world. China presently produces the majority of the world's peaches, which are predominantly sold in the domestic market, while in Pakistan, it is the second largest produced stone fruit after apricots [1]. In Pakistan, peaches constitute a fundamental source of livelihood for thousands of farmers in the provinces of Khyber Pukhtunkhwa and Baluchistan as well as in Pothwar zone of Punjab province [2]. It has been cultivated on an area of 14,700 hectares with 55,800 tons of production [3]. The lucrative production of peaches and nectarines has been threatened for many years by an assortment of biotic factors including diseases, like peach leaf curl and peach tree short life (PTSL), and nematodes [4–12]. However, phytopathogenic nematodes have economic significance in stone fruits' production and revenue generation. Globally,

these nematodes have been reported to incur a colossal loss of over USD 173 billion annually on different agricultural crops. The root-knot nematodes of the genus *Meloidogyne* are the most damaging ones and ranked on top of all phytopathogenic nematodes [13–15].

Over 100 species of *Meloidogyne* have been explored worldwide thus far, which have been found distributed in temperate, tropical, and equatorial regions of the world [16–19]. Root-knot nematodes have been found seriously infecting peaches and have become a severe issue for the majority of peach growers and nurserymen in many regions having tropical and Mediterranean climates [13,20–22]. Root-knot nematodes cause reduction in fruit production of many economically important species of *Prunus* including *Prunus persica*. Among different species of root-knot nematodes, *Meloidogyne incognita* and *M. javanica* are the most common in peach and plum orchards [22,23]. In Pakistan, both the species are predominant as sole and mixed populations [19,24]. Several methods are used to control root-knot nematodes [25–37], but growers frequently utilize chemical nematicides because other management methods have some specific drawbacks [38]. The usage of nematicides, however, is frequently linked to health risks and environmental contamination. Due to the broad spectrum activities of most of the pernicious chemical nematicides, beneficial soil microbes are badly affected resulting in a rapid resurgence of soil-dwelling phytopathogens. As a result, the development of new, safer, and environmentally friendly methods for managing root-knot nematodes has become essential in order to achieve sustainable development goals of zero hunger, poverty alleviation, and environment protection especially in developing countries of South Asia.

Among various substitutes used to phase out the use of chemical nematicides, deployment of biological control agents is considered as one of the feasible options. These agents can be used singly or in combinations with other nematode management approaches. A myriad of biological control agents have been reported to be effective against root-knot nematodes [39–43]. One of the fungal biocontrol agents, *Pochonia chlamydosporia*, which parasitizes females and eggs of root-knot nematode, is distributed worldwide. It has shown a great potential for the effective management of root-knot nematodes [44,45]. Another fungus, *Purpureocillium lilacinum*, infecting ova and females of root-knot nematodes, has also been found effective in causing mortalities of embryos of nematodes in 5 to 7 days [39,46,47]. Similarly, *Trichoderma harzianum* and *T. viride* have been extensively investigated throughout the world and proved effective in the management of root-knot and many other plant pathogenic nematodes [43]. These fungal biocontrol agents have different mechanisms of action to maintain the nematode populations at low levels; however, their efficacy tend to reduce under severe attack of nematodes that necessitates conducting fresh studies to assess and sort out more effective biocontrol agents.

As scientific information available pertaining to the effectiveness of biocontrol agents (*P. chlamydosporia*, *P. lilacinum*, *T. harzianum,* and *T. viride*) against root-knot nematodes on peach is scant, therefore, the present investigations were carried out to assess the comparative effectiveness of these biocontrol agents with an ultimate aim to find out a biologically viable management option for *M. javanica* in peach.

2. Materials and Methods

2.1. Preparation of Inoculum of M. javanica

The biocontrol agents (*Pochonia chlamydosporia*, *Purpureocillium lilacinum*, *Trichoderma harzianum,* and *T. viride*) were tested against the nematode, *M. javanica*, which was obtained from a single egg mass and mass produced on a highly susceptible tomato cultivar (money maker) as described by Zhang et al. [48]. The eggs were obtained from the infected roots by treating them with 300 mL of 1% sodium hypochlorite (NaOCl) in a tightly closed bottle. The roots in the bottle were shaken vigorously for the dissolution of gelatinous egg masses and release of eggs. The resultant suspension was passed through 150 µm sieve to remove roots and then through 38 µm to collect eggs. The eggs were then rinsed using a 38 µm sieve to wash off residues of the bleach and back-washed in a beaker. The eggs were then placed on extraction trays for emergence of second-stage juveniles. The freshly hatched

(24–48 h old) juveniles thus obtained were used in the evaluation of fungal biocontrol agents in pot experiments.

2.2. Soil Used for the Experiment

A soil comprising of 55% sand, 20% silt, 24% clay, and 1% organic matter with pH of 7.6 was used in the assay. The soil was sifted through a 3.5 mm mesh sieve to remove pebbles and plant residues. The soil was sterilized with formalin and filled in pots.

2.3. Evaluation of Biocontrol Agents for Their Effectiveness against M. Javanica

2.3.1. Mass Production of Biocontrol Agents

Pochonia chlamydosporia

Barley seeds were crushed, washed over a 53 µm mesh sieve, and blot dried. These were then blended with rough sand in a 1:1 (*v*/*v*) ratio and left to dry partly until readily crumbled. A 200 mL volume of this culture medium was added into 500 mL conical flasks, autoclaved at 15 psi for 30 min, and then allowed to cool with shaking. The autoclaved media in the flasks were inoculated with five 7 mm diameter plugs of purified culture of *P. chlamydosporia* grown on corn meal agar and incubated for three weeks at 25 °C. The flasks were shaken on alternate days for uniform colonization of the fungus. Three weeks after incubation, the colonized sand/bran medium was cleansed using sieves of 250, 53, and 10 µm mesh using a gentle stream of water to wash off sand and bran. The fungal propagules were then collected on a sieve of 10 µm mesh. The deposited material on the sieve was further rinsed to wash off conidia and small hyphal fragments, and the chlamydospores left on the sieve were blotted dry to remove extra moisture. Chlamydospores were scraped off from the surface of the sieve and mixed with fine sand (40–100 mesh) in a ratio of 1:10 (*w*/*w*), which served as an inert carrier. A subsample of 1 g from this inoculum was dissolved in 9 mL of water, and the number of chlamydospores per g of sand was counted with the help of a hemocytometer.

Purpureocillium lilacinum, *Trichoderma harzianum*, and *T. viride*

The inocula of *P. lilacinum*, *T. harzianum,* and *T. viride* were mass produced on ground wheat grains. For this purpose, chopped wheat grains were soaked in water for approximately 12 h and blotted dry, and 250 g of these dried wheat grains were added into separate 500 mL flasks. The grains in the flasks were autoclaved at 15 psi for about 50 min and allowed to cool down. The sterilized wheat grains in flasks were inoculated separately with five 7 mm diameter plugs of purified cultures each of *P. lilacinum*, *T. harzianum,* and *T. viride* grown on Potato Dextrose Agar. The inoculated flasks were then incubated at 25 ± 1 °C for about 2 weeks. The flasks were agitated after every two days for uniform colonization of the fungus. For counting spores, 1 g subsample from each colonized flasks were mixed with 9 mL of water to make a spore suspension. Spores per g of the grains were counted using a hemocytometer.

2.4. Evaluation of the Efficacy of Biocontrol Agents

The effectiveness of biocontrol agents viz. *P. chlamydosporia*, *P. lilacinum*, *T. harzianum,* and *T. viride* against *M. Javanica* was assessed in a pot experiment. Each biocontrol agent was mixed with formalin-sterilized soil at the rates of 2.5×10^3, 5×10^3, 7.5×10^3, and 1×10^4 chlamydospores or cfu per g of soil. Five kg of the biocontrol-amended soils were then filled in earthen pots separately. The pots without any biocontrol agent were kept as controls. For comparison with standard nematicide, Rugby was used. One week after treatments, one healthy peach plant cv. 'Early Grand', which was one year old, was transplanted in each pot. The plants were allowed to grow for two weeks to establish roots in the soil. Two weeks after transplantation, the peach plantlets were inoculated with 5000 freshly hatched (24–48 h of age) second-stage juveniles of *M. Javanica*. There were five replications for each treatment. The pots were arranged following a completely

randomized design in a glasshouse at 25 °C ± 2 for seven weeks. The pots were irrigated when required.

2.5. Data Recordings

After specified period, the plants were carefully removed, the roots were excised from the shoots, carefully washed under tap water, and blotted dry. The data were recorded regarding number of galls, egg masses, eggs per egg mass, root, soil, total population, and reproduction factor of the nematode. The reproduction factor was calculated by the following formula:

$$\text{Reproduction factor} = (\text{Final population})/(\text{Initial population})$$

The percent reductions in these variables were calculated compared with control as mentioned below:

$$\text{Reduction over control (\%)} = (\text{uninoculated} - \text{inoculated})/\text{uninoculated} \times 100$$

2.6. Statistical Analyses

All the data were subjected to analysis of variance (ANOVA) using Statistix 8.1 package. The means were compared by Tukey's Honestly Significant Difference Test HSD using the SAS statistical package (9.2 Version, SAS Institute, Cary, NC, USA) at 0.05%. Standard errors of means were calculated in Microsoft Excel 2016.

3. Results

3.1. Effect of Biocontrol Agents on Number of Galls

The analysis of variance regarding effects of biocontrol agents on number of galls showed significant effects of biocontrol agents and their concentrations ($p < 0.01$). All the four fungal biocontrol agents caused reductions in number of galls. The overall maximum reductions were caused by *T. harzianum* and *P. lilacinum* followed by *P. chlamydosporia*, while *T. viride* resulted in the minimum reduction in number of galls. All the biocontrol agents caused reductions in galls in a dose-dependent manner. In the control treatment where only nematodes were applied, the maximum galls were observed, while the highest dose of 10^4 spores/mL produced the minimum number of galls. When compared with nematicide, Rugby reduced galls to the maximum level. The number of galls in each treatment at each dose is given in Table 1, while their reductions compared with control are given in Table 2.

Table 1. Effectiveness of biocontrol agents on the number of galls produced by *M. javanica* on peach.

Concentration	*Pochonia chlamydosporia*	*Purpureocillium lilacinum*	*Trichoderma harzianum*	*Trichoderma viride*
0	218 ± 19.13 ab	218 ± 19.13 ab	218 ± 19.13 ab	218 ± 19.13 ab
2.5×10^3	210 ± 10.00 ab	202 ± 25.88 abc	214 ± 18.92 ab	232 ± 20.63 a
5×10^3	204 ± 10.20 abc	198 ± 17.44 abc	190 ± 18.97 bc	224 ± 17.61 ab
7.5×10^3	188 ± 23.87 bc	168 ± 24.21 cd	166 ± 11.85 cd	192 ± 23.02 abc
1×10^4	142 ± 23.71 de	124 ± 19.03 e	120 ± 13.29 e	136 ± 18.67 de
Rugby	52 ± 7.00 f	52 ± 7.00 f	52 ± 7.00 f	52 ± 7.00 f

± depicts standard deviation while different letters within the same column exhibit significant difference at $p = 0.05\%$.

Table 2. Percent reductions in number of galls produced by *M. javanica* on peach by biocontrol agents, compared with the control.

Concentration	*Pochonia chlamydosporia*	*Purpureocillium lilacinum*	*Trichoderma harzianum*	*Trichoderma viride*
0	0.00	0.00	0.00	0.00
2.5×10^3	2.11	5.63	4.23	−0.70
5×10^3	6.34	7.75	7.04	2.11
7.5×10^3	11.27	11.97	9.15	8.45
1×10^4	16.20	18.31	16.20	14.08
Rugby	21.83	21.83	21.83	21.83

3.2. Effect of Biocontrol Agents on Number of Egg Masses

The analysis of variance showed non-significant results for biocontrol agents ($p > 0.05$), while their concentrations and their interactions were found to be significant ($p < 0.05$) vis à vis effects of biocontrol agents on number of egg masses. Among treatments, the minimum numbers of egg masses were observed where Rugby was applied. Similarly, all the biocontrol agents resulted in reductions in numbers of egg masses. Among concentrations, the maximum reductions were caused by the highest doses of the biocontrol agents (Tables 3 and 4).

Table 3. Effectiveness of biocontrol agents on the number of egg masses produced by *M. javanica* on peach.

Concentration	*Pochonia chlamydosporia*	*Purpureocillium lilacinum*	*Trichoderma harzianum*	*Trichoderma viride*
0	210 ± 13.78 a	210 ± 13.78 a	210 ± 13.78 a	210 ± 13.78 a
2.5×10^3	192 ± 21.86 abc	182 ± 24.12 abcd	186 ± 17.00 abcd	198 ± 17.44 ab
5×10^3	180 ± 17.89 abcd	162 ± 12.51 bcdef	164 ± 11.90 bcde	182 ± 20.74 abcd
7.5×10^3	152 ± 10.07 defg	156 ± 16.67 cdefg	138 ± 19.24 efg	148 ± 23.71 defg
1×10^4	130 ± 21.94 efg	128 ± 16.54 efg	122 ± 18.25 g	124 ± 20.98 fg
Rugby	44 ± 9.38 h	44 ± 9.38 h	44 ± 9.38 h	44 ± 9.38 h

± depicts standard deviation while different letters within the same column exhibit significant difference at $p = 0.05\%$.

Table 4. Percent reductions in the number of egg masses produced by *M. javanica* on peach by biocontrol agents, compared with the control.

Concentration	*Pochonia chlamydosporia*	*Purpureocillium lilacinum*	*Trichoderma harzianum*	*Trichoderma viride*
0	0.00	0.00	0.00	0.00
2.5×10^3	8.57	13.33	11.43	5.71
5×10^3	14.29	22.86	21.90	13.33
7.5×10^3	27.62	25.71	34.29	29.52
1×10^4	38.10	39.05	41.90	40.95
Rugby	79.05	79.05	79.05	79.05

3.3. Effect of Biocontrol Agents on the Number of Eggs Per Egg Mass

Likewise, as far as the number of eggs per egg mass were concerned, the analysis of variance provided an almost similar result as observed in the case of the number of egg masses. The maximum number of eggs per egg mass was produced by females of *M. javanica* where no biocontrol agent was applied, while the minimum number was recorded in the case of Rugby. Similarly, among biocontrol agents, the maximum reduction

in the number of eggs was obtained at the highest dose of biocontrol agents, i.e., 10^4, which was at par with Rugby. The number of eggs produced by females were found to be dose dependent. The number of eggs per egg mass in each treatment and at each dose are shown in Table 5, and the percent reductions compared with control are given in Table 6.

Table 5. Effectiveness of biocontrol agents on the number of eggs per egg mass produced by *M. javanica* on peach.

Concentration	*Pochonia chlamydosporia*	*Purpureocillium lilacinum*	*Trichoderma harzianum*	*Trichoderma viride*
0	284 ± 16.79 a	284 ± 16.79 a	284 ± 16.79 a	284 ± 16.79 a
2.5×10^3	278 ± 20.83 ab	268 ± 19.44 abcd	272 ± 14.35 abc	286 ± 17.72 a
5×10^3	266 ± 12.81 abcd	262 ± 12.88 abcd	264 ± 15.03 abcd	278 ± 16.06 ab
7.5×10^3	252 ± 14.63 abcde	250 ± 14.14 abcde	258 ± 17.72 abcde	260 ± 21.91 abcde
1×10^4	238 ± 21.31 cde	232 ± 12.00 de	238 ± 14.28 cde	244 ± 9.70 bcde
Rugby	222 ± 14.83 e	222 ± 14.83 e	222 ± 14.83 e	222 ± 14.83 e

± depicts standard deviation while different letters within the same column exhibit significant difference at $p = 0.05\%$.

Table 6. Percent reductions in eggs per egg mass produced by *M. javanica* on peach by biocontrol agents, compared with the control.

Concentration	*Pochonia chlamydosporia*	*Purpureocillium lilacinum*	*Trichoderma harzianum*	*Trichoderma viride*
0	0.00	0.00	0.00	0.00
2.5×10^3	2.11	5.63	4.23	−0.70
5×10^3	6.34	7.75	7.04	2.11
7.5×10^3	11.27	11.97	9.15	8.45
1×10^4	16.20	18.31	16.20	14.08
Rugby	21.83	21.83	21.83	21.83

3.4. Effect of Biocontrol Agents on Populations of M. javanica

The biocontrol agents and their concentrations caused significant reductions in total populations of the nematode ($p < 0.05$). The reductions caused by Rugby were found to be the maximum amongst all the treatments. The biocontrol agents caused reductions in a dose-responsive manner. The reductions were found to be the maximum at the highest dose. With an increase in the dose of the biocontrol agent, there was a corresponding decrease in the populations of the nematode and the relationships were found to be inversely proportional. The populations of the nematode and their corresponding decreases over control are shown in Tables 7 and 8.

Table 7. Effectiveness of biocontrol agents on the total population of *M. javanica*.

Concentration	*Pochonia chlamydosporia*	*Purpureocillium lilacinum*	*Trichoderma harzianum*	*Trichoderma viride*
0	61,510 ± 4915 a	61,510 ± 4917 a	61,510 ± 4915 a	61,510 ± 4917 a
2.5×10^3	55,078 ± 9271 abc	49,862 ± 4617 abcde	51,998 ± 5387 abcd	58,346 ± 7832 ab
5×10^3	48,988 ± 3620 bcdef	43,582 ± 4479 cdefg	44,466 ± 4355 cdef	51,612 ± 3249 abcd
7.5×10^3	39,316 ± 2045 efgh	40,052 ± 5388 defgh	36,776 ± 6530 fgh	39,832 ± 8550 defgh
1×10^4	31,912 ± 6194 gh	30,678 ± 4920 h	29,896 ± 4553 h	31,312 ± 5747 gh
Rugby	10,590 ± 2257 i	10,590 ± 2258 i	10,590 ± 2257 i	10,590 ± 2257 i

± depicts standard deviation while different letters within the same column exhibit significant difference at $p = 0.05\%$.

Table 8. Percent reductions in the total population of *M. javanica* by biocontrol agents, compared with the control.

Concentration	*Pochonia chlamydosporia*	*Purpureocillium lilacinum*	*Trichoderma harzianum*	*Trichoderma viride*
0	0.00	0.00	0.00	0.00
2.5×10^3	10.46	18.94	15.46	5.14
5×10^3	20.36	29.15	27.71	16.09
7.5×10^3	36.08	34.89	40.21	35.24
1×10^4	48.12	50.13	51.40	49.09
Rugby	82.78	82.78	82.78	82.78

3.5. Effect of Biocontrol Agents on the Reproductive factors of M. javanica

Significant effects of the various concentrations of biocontrol agents ($p < 0.05$) were found on the reproductive factor of *M. javanica*, while the effects were non-significant in case of biocontrol agents and their interactions ($p > 0.05$). Application of biocontrol agents in the soil at all concentrations resulted in significant reductions in reproduction factor. Reductions were found to be the maximum at an application rate of 10^4 spores/mL. Rugby, with which the effectiveness of biocontrol agents was compared, resulted in the maximum reduction of reproductive factor. It was observed that as the concentration of the biocontrol agent increased, there was a corresponding decrease in the reproduction factors, which were found to be inversely proportional to the doses of the biocontrol agents. Individual reproductive factors at each concentration of the biocontrol agents and their respective percent decreases are shown in Tables 9 and 10.

Table 9. Effectiveness of biocontrol agents on the reproductive factor.

Concentration	*Pochonia chlamydosporia*	*Purpureocillium lilacinum*	*Trichoderma harzianum*	*Trichoderma viride*
0	12.302 ± 0.98 a	12.302 ± 0.98 a	12.302 ± 0.98 a	12.302 ± 0.98 a
2.5×10^3	11.016 ± 1.85 abc	9.972 ± 0.92 abcde	10.400 ± 1.08 abcd	11.669 ± 1.57 ab
5×10^3	9.798 ± 0.72 bcdef	8.716 ± 0.90 cdefg	8.893 ± 0.87 cdef	10.322 ± 0.65 abcd
7.5×10^3	7.863 ± 0.41 efgh	8.010 ± 1.08 defgh	7.355 ± 1.31 fgh	7.966 ± 1.71 defgh
1×10^4	6.382 ± 1.24 gh	6.136 ± 0.98 h	5.979 ± 0.91 h	6.262 ± 1.15 gh
Rugby	2.118 ± 0.45 i	2.118 ± 0.45 i	2.118 ± 0.45 i	2.118 ± 0.45 i

± depicts standard deviation while different letters within the same column exhibit significant difference at $p = 0.05\%$.

Table 10. Percent reductions in reproductive factors by biocontrol agents, compared with the control.

Concentration	*Pochonia chlamydosporia*	*Purpureocillium lilacinum*	*Trichoderma harzianum*	*Trichoderma viride*
0	0.00	0.00	0.00	0.00
2.5×10^3	10.41	18.94	15.45	5.12
5×10^3	20.33	29.11	27.72	16.10
7.5×10^3	36.10	34.88	40.16	35.20
1×10^4	48.13	50.08	51.38	49.11
Rugby	82.76	82.76	82.76	82.76

4. Discussion

Recently, for the control of root-knot nematodes in peaches, several management options and strategies are being practiced, but the use of chemical nematicides remains

the most widely adopted option despite having numerous limitations [2,5,22,25,30,32–34]. Due to broad spectrum activities of most of the pernicious chemical nematicides, beneficial soil microbes are adversely affected resulting in a rapid resurgence of soil-dwelling phytopathogens [49]. Thus, the development of new, safer, ecofriendly nematicidal chemicals for the management of root-knot nematodes has become essential to enhance peach productivity and increase the net returns for peach growers. Among various substitutes used to phase out the use of chemical nematicides, deployment of biological control agents might be considered a feasible option. These biocontrol agents can be used solely or in combinations with other nematode management approaches. A myriad of biological control agents have been reported to be effective against root-knot nematodes [39–43,50,51], but their effectiveness against root-knot nematodes of peach has not been studied. Therefore, four biocontrol agents viz. *P. chlamydosporia*, *P. lilacinum*, *T. harzianum,* and *T. viride* were comparatively assessed for their efficacy against *M. javanica* on peach. According to our results, all biocontrol agents exhibited dose-dependent reductions in nematode infestations, including the number of galls, egg masses, nematode populations, and reproductive factors as compared to those achieved by the nematicide Rugby.

The reductions in different nematode infestation criteria caused by *P. lilacinum* were found to be at par with other fungal biocontrol agents. The fungal biocontrol agent, which is a good root colonizer and rhizosphere competitor, has been extensively studied for its potential as a biocontrol agent. It has successfully controlled and inhibited many plant parasitic nematodes, resulting in yield enhancements [39,46,47,52]. The underlying mechanism of action involves the colonization of gelatinous matrices of *Meloidogyne* species by the fungus, which forms a mycelial network and engulfs nematode eggs. The fungus then penetrates the nematode mechanically as well as enzymatically with the help of appressoria or simple hyphae [39]. These findings are in agreement with those of Morgan-Jones et al. [53] who reported that penetration of eggshells of *M. arenaria* by fungal hyphae resulted through small pores dissolved in the vitelline layer. After entering, the growth and proliferation of the fungus starts in the eggs, which are in their early embryonic development. When all the nutrients in the eggs are consumed, the mycelia enter and break the eggshells and come out to cause infections in the eggs found in the surroundings. The fungus has also been known to colonize juveniles found in the eggshells as well as the third and fourth larval stages of the nematode on water agar [54].

Toxicity of culture filtrates of *P. lilacinus* has also been proved against nematodes [55,56]. When nematodes were exposed to different concentrations of culture filtrates of *P. lilacinus*, their cuticles were ruptured causing deaths of nematodes within few hours [55]. Culture filtrates of the fungus contain chemicals that have been identified and characterized. One of such chemicals, a peptidal antibiotic designated as P-168, has been isolated and identified and has been proved to have antimicrobial activity against a number of pathogens [57].

The present findings also proved the effectiveness of *P. chlamydosporia* in reducing infestations by *M. javanica* on peach. The reductions caused by *P. chlamydosporia* were statistically not different from other biocontrol agents. *P. chlamydosporia* (*Verticillium chlamydosporium*), similar to *P. lilacinus*, has the potential to parasitize ova and adult females of root-knot nematodes [53,58]. Although *P. chlamydosporia* has been recognized as a parasite of eggs of nematodes, it can produce branched mycelial networks, which can beset eggshells of nematodes [53,59,60]. The fungus enters the eggshells by making specialized structures known as penetration pegs or appressoria. The fungus also produces lateral branches of mycelia and results in the dissolution of chitin, lipid, and vitelline layers of eggshells [61]. Enzymes such as proteases and chitinases are known to be involved in the initiation of infection process [62]. Different studies have also verified that isolates of *P. chlamydosporia* can produce a variety of different subtilisins [62].

In a previous study, *P. chlamydosporia* was identified as the least effective among the four evaluated biocontrol agents against *M. incognita*. However, the fungus exhibited significant improvements in plant growth variables, which were not statistically different from the other tested biocontrol agents. This decrease in the effectiveness of *P. chlamy-*

dosporia was attributed to the use of an exotic population of the fungus, which was not found effective against the indigenous population of *M. incognita* [63]. However, in the present study, an indigenous isolate of the fungus was used against a different nematode species, i.e., *M. javanica*. It has been proved that different isolates of *P. chlamydosporia* have shown variations in aggressiveness against different root-knot populations. Likewise, differences in parasitism by *P. chlamydosporia* isolates have also been noticed among root-knot populations [64].

In the present assessment, both the species of *Trichoderma* caused reductions in root galling, egg masses, fecundity, and reproductive factor of *M. javanica*. Many earlier studies have shown good control of root-knot nematodes and many plants pathogenic fungi by different species of *Trichoderma* [65–68]. *Trichoderma* species being omnipresent dwell freely in all types of soils and are frequently found in root ecosystems of plants. These opportunistic fungi grow symbiotically and develop associations with various kinds of plants and fungal species. Certain species of *Trichoderma* possess the capability to infect and grow on the root surfaces of plants and can penetrate the epidermal cells [69]. As a result, the roots grow and develop profusely resulting in an increase in productivity and resistance towards an assortment of abiotic constraints, and the ability of the plants to imbibe minerals and nutrients from the soil is significantly bettered. Likewise, the biocontrol potential of *Trichoderma* species against plant pathogens can be ascribed to numerous mechanisms. Among these vital mechanisms, production of antibiotics, competition, enzymatic hydrolysis, parasitism, and induction of systemic resistance are considered noteworthy [70,71]. A large number of biocontrol substances, catalysts, and activators like trichokonins, trichodermin, and trypsin-like protease have been reported to be produced by different species of *Trichoderma* and have shown nematostatic and nematicidal activities against many nematode species [72–74].

The extensively branched conidiophores of *Trichoderma* produce conidia, which can stick to different nematode developmental stages. Attachment of conidia to nematode and parasitism differs amongst fungal species and strains [65]. This process of infection is frequently initiated with the development of fungal coiling and formation of appressorium-like structures. *T. harzianum* colonizes isolated eggs and second-stage juveniles of *M. javanica* [65]. For effective infection and parasitism of nematodes by *Trichoderma* species, mechanisms for facilitation of entrance of the cuticles and/or eggshells of nematodes by the fungus are essential. Lytic enzymes have been reported to be involved in the infection and parasitism of *Meloidogyne* [75]. In addition to direct antagonism, several other mechanisms and processes including secretion of various metabolites and induction of resistance in host plants have also been found involved in the management of *Meloidogyne* species by different species of *Trichoderma* [76]. Decrease in nematode galling and fecundity might be a result of high rhizosphere capability of biocontrol agents as they can readily infect and colonize roots and may decrease feeding sites for nematodes. The decrease in number of galls may also be a result of the inability of a large number of nematodes to penetrate the roots of plants. It is generally recommended that *Trichoderma* should be used prior to planting of host plants in order to allow the fungus to establish efficiently in the soil or rhizospheres of plants for obtaining greatest nematode control. These research findings might serve as a basis to conduct further studies for evaluating more biocontrol agents and their doses for developing biocontrol agents-based management option for *M. javanica* in peach.

5. Conclusions

The present study explored the comparative efficacy of four biocontrol agents, namely, *P. chlamydosporia*, *P. lilacinum*, *T. harzianum*, and *T. viride*, against *M. javanica* that infects peach. Peach growers in the region have consistently faced nematode attacks, which necessitated the testing of farmer-friendly and environmentally beneficial strategies such as biocontrol agents. The study demonstrated that biocontrol agents can act effectively as nematicides. They can be successfully used to control root-knot nematodes, potentially

replacing or reducing the need for traditional chemical treatments and mitigating environmental pollution. However, future research needs to investigate the impact of combining these biocontrol agents with reduced doses of chemical treatments to develop an efficient and biologically feasible control approach for *M. javanica* in peach.

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References

1. Manganaris, G.A.; Minas, I.; Cirilli, M.; Torres, R.; Bassi, D.; Costa, G. Peach for the future: A specialty crop revisited. *Sci. Hortic.* **2022**, *305*, 111390. [CrossRef]
2. Saeed, M.; Mukhtar, T.; Haq, M.I.; Khan, M.A. Assessment of nematicidal potential of *Cannabis sativa* and *Azadirachta indica* in the management of root-knot nematode (*Meloidogyne javanica*) on peach. *Pak. J. Agric. Sci.* **2021**, *58*, 1555–1561.
3. FAO. Statistical Database-Agriculture. 2016. Available online: http://faostat3.fao.org (accessed on 31 August 2023).
4. Ahmad, Z.; Abbas, H.; Murtaza, T.; Khan, A.U.R.; Ali, A.; Zahid, K.; Tahir, Z.; Mahmood, T.; Habib, A. Assessment of responses of peach cultivars to postharvest pathogen *Botrytis cinerea* and its mitigation using plant essential oils. *Plant Prot.* **2023**, *7*, 153–162.
5. Eliwa, G.I.; Hagag, E.S. Approach to new peach rootstocks resistant to root-knot nematodes (*Meloidogyne* species) selected from local Mit-Ghamer peach cultivar. *Sci. Hortic.* **2021**, *284*, 110118. [CrossRef]
6. Haq, M.A.; Mukhtar, T.; Haq, M.I.; Khalid, A. Reproduction of root-knot nematode, *Meloidogyne incognita*, on *Solanum melongena* genotypes determines their host status. *Pak. J. Zool.* **2022**, *54*, 2097–2103. [CrossRef]
7. Luo, C.X.; Schnabel, G.; Hu, M.; De Cal, A. Global distribution and management of peach diseases. *Phytopathol. Res.* **2022**, *4*, 30. [CrossRef]
8. Mahmood, B.; Saeed, M.; Rehman, A.U.; Saleem, S.; Khan, M.R.; Younas, M.T.; Shafqat, M.; Khan, Z.H.; Hussain, S. Surveillance of bacterial canker of peach in Azad Jammu and Kashmir, and its bio-management. *Plant Prot.* **2023**, *7*, 83–91. [CrossRef]
9. Mahmood, T.; Moosa, A.; Khan, A.U.R.; Maqsood, A.; Abbas, G.; Alyas, K.; Khalid, B. Using essential oils to protect peaches from post-harvest rot caused by *Rhizopus* species. *Plant Prot.* **2023**, *7*, 217–223.
10. Ali, I.; Wang, X.; Tareen, M.J.; Wattoo, F.M.; Qayyum, A.; Hassan, M.U.; Shafique, M.; Liaquat, M.; Asghar, S.; Hussain, T.; et al. Foliar Application of Salicylic Acid at Different Phenological Stages of Peach Fruit CV. 'Flordaking' Improves Harvest Quality and Reduces Chilling Injury during Low Temperature Storage. *Plants* **2021**, *10*, 1981. [CrossRef]
11. Manzoor, M.; Anwar, F.; Mahmood, Z.; Rashid, U.; Ashraf, M. Variation in Minerals, Phenolics and Antioxidant Activity of Peel and Pulp of Different Varieties of Peach (*Prunus persica* L.) Fruit from Pakistan. *Molecules* **2012**, *17*, 6491–6506. [CrossRef]
12. Khalil, M.; Alqadasi, A. Potential of Non-Fumigant Nematicides at Different Formulations Against Southern Root-knot Nematode (*Meloidogyne incognita*) on Tomato Plants. *Int. J. Phytopathol.* **2019**, *8*, 23–28. [CrossRef]
13. Nyczepir, A.P.; Beckman, T.G. Host status of guardian peach rootstock to *Meloidogyne* sp. and *M. javanica*. *Hortscience* **2000**, *35*, 772. [CrossRef]
14. Shahid, M.; Gowen, S.R.; Burhan, M. Studies on the possible role of plant host on the development of root-knot nematode, *Meloidogyne javanica* and *Pasteuria penetrans* as affected by different harvesting dates. *Plant Prot.* **2022**, *6*, 133–141. [CrossRef]
15. Shahid, M.; Gowen, S.R.; Burhan, M.; Niaz, Z.; Haq, A. Studies on the efficacy of heterogeneously produced *Pasteuria penetrans* (PP3) isolate over individual *Pasteuria* isolates in the spore attachment, and pathogenic potential on three *Meloidogyne* species. *Plant Prot.* **2023**, *7*, 9–16. [CrossRef]
16. Elling, F.A. Major emerging problems with minor *Meloidogyne* species. *Phytopathology* **2013**, *103*, 1092–1102. [CrossRef]
17. Moens, M.; Perry, R.N.; Starr, J.L. *Meloidogyne Species—A Diverse Group of Novel and Important Plant Parasites, Root-Knot Nematodes*; CAB International: Wallingford, UK, 2009; pp. 1–17.

18. Ahamad, L.; Siddiqui, M. Efficacy of Botanicals and Carbofuran for the Control of Meloidogyne incognita Affecting *Solanum lycopersicum* L. *Int. J. Phytopathol.* **2018**, *7*, 69–75. [CrossRef]
19. Azeem, W.; Mukhtar, T.; Hamid, T. Evaluation of *Trichoderma harzianum* and *Azadirachta indica* in the management of *Meloidogyne incognita* in Tomato. *Pak. J. Zool.* **2021**, *53*, 1–7. [CrossRef]
20. Nyczepir, A.P.; Wood, B.W.; Reighard, G.L. Impact of *Meloidogyne incognita* on the incidence of peach tree short life in the presence of *Criconemella xenoplax*. *J. Nematol.* **1997**, *29*, 725–730.
21. Stassen, P.J.C.; Malan, A.P. Host suitability of commercial peach and plum rootstocks to plant-parasitic nematodes. *Acta Hortic. Int. Soc. Hort. Sci.* **2021**, *1304*, 315–320. [CrossRef]
22. Esmenjaud, D. Deciphering resistance to root-knot nematodes in *Prunus* for rootstock breeding: Sources, genetics and characterization of the ma locus. *Horticulturae* **2021**, *7*, 564. [CrossRef]
23. Nyczepir, A.P.; Becker, J.O. Fruit and citrus trees. *Plant Nematode Interact.* **1998**, *36*, 637–684.
24. Bakr, R. Nematicidal activity of jimson weed (*Datura* spp.) for management of plant-parasitic nematodes with emphasis on root knot nematode: A review. *Pak. J. Phytopathol.* **2021**, *33*, 183–204. [CrossRef]
25. Afzal, A.; Ahmad, A.; Hassaan, M.A.; Mushtaq, S.; Abbas, A. Enhancing agricultural sustainability in Pakistan: Addressing challenges and seizing opportunities through effective plant disease management. *Plant Prot.* **2023**, *7*, 341–350.
26. Alam, E.A.A.; Nuby, A.S.E. Phytochemical and nematicidal screening on some extracts of different plant parts of egyptian *Moringa oleifera* L. *Pak. J. Phytopathol.* **2022**, *34*, 293–306. [CrossRef]
27. Khan, M.T.A.; Mukhtar, T.; Saeed, M. Resistance or susceptibility of eight aubergine cultivars to *Meloidogyne javanica*. *Pak. J. Zool.* **2019**, *51*, 2187–2192. [CrossRef]
28. Khan, R.A.; Atiq, M.; Rajput, N.A.; Ahmad, I.; Husnain, A.; Nawaz, A.; Mehtab, M.; Ahmad, W. Assessment of phytoextracts and synthetic chemicals for controlling leaf blight of *Syzygium cumini*. *Plant Prot.* **2023**, *7*, 193–205.
29. Mehmood, B.; Jamil, M.; Shafique, S.; Khan, M.R.; Zafar, T.; Bhatti, R.M.; Qayyum, H.; Tasneem, K.; Younas, M.T.; Bakar, M.A. Evaluating the biocontrol efficacy of selected botanical extracts against bacterial spot of tomato. *Plant Prot.* **2023**, *7*, 255–261.
30. Meyer, S.L.F.; Nyczepir, A.P.; Rupprecht, S.M.; Mitchell, A.D.; Martin, P.A.W.; Brush, C.W.; Chitwood, D.J.; Vinyard, B.T. Tall fescue 'Jesup (max-Q)': *Meloidogyne incognita* development in roots and nematotoxicity. *Agron. J.* **2013**, *105*, 755–763. [CrossRef]
31. Mukhtar, T.; Tariq-Khan, M.; Aslam, M.N. Bioefficacy of *Trichoderma* species against Javanese root-knot nematode, *Meloidogyne javanica* in green gram. *Gesunde Pflanz.* **2021**, *73*, 265–272. [CrossRef]
32. Nunes, L.V.; Buonicontro, D.S.; Rosado, L.D.S.; Fonseca, A.R.; Bruckner, C.H.; dos Santos, C.E.M. Selection of genotypes of peach rootstock resistant to *Meloidogyne incognita*. *Rev. Ceres* **2022**, *69*, 267–273. [CrossRef]
33. Nyczepir, A.P.; Kluepfel, D.A.; Waldrop, V.; Wechter, W.P. Soil solarization and biological control for managing *Mesocriconema xenoplax* and short life in a newly established peach orchard. *Plant Dis.* **2012**, *96*, 1309–1314. [CrossRef] [PubMed]
34. Rajput, P.; Thakur, A.; Singh, H.; Singh, J.; Kaur, S.; Koulagi, R.; Pathak, D. Screening of peach rootstock hybrids for resistance to root-knot nematode. *Indian J Hortic.* **2021**, *78*, 134–141. [CrossRef]
35. Shahbaz, M.; Akram, A.; Raja, N.I.; Mukhtar, T.; Mehak, A.; Fatima, N.; Ajmal, M.; Ali, K.; Mustafa, N.; Abasi, F. Antifungal activity of green synthesized selenium nanoparticles and their effect on physiological, biochemical, and antioxidant defense system of mango under mango malformation disease. *PLoS ONE* **2023**, *18*, e0274679. [CrossRef]
36. Shirley, A.M.; Noe, J.P.; Nyczepir, A.P.; Brannen, P.M.; Shirley, B.J.; Jagdale, G.B. Effect of spirotetramat and fluensulfone on population densities of *Mesocriconema xenoplax* and *Meloidogyne incognita* on peach. *J. Nematol.* **2019**, *51*, 1–10. [CrossRef] [PubMed]
37. Talpur, M.S.A.; Abro, M.A.; Jatoi, G.H.; Marri, J.M.; Poussio, G.B.; Qayoom, M.M.; Rajer, F.U.; Hajano, J.; Shafique, S. Exploring the effects of plant extracts, biological agents and essential oils on the non-chemical management of banana leaf spot disease. *Plant Prot.* **2023**, *7*, 321–330.
38. Khan, M.; Ahmad, I.; Atiq, M.; Asif, M.; Rashid, M.H.U.; Ahmad, S.; Shaheen, H.M.F.; Adil, Z. Comparative assessment of various antibiotics for controlling bacterial blight in *Eucalyptus camaldulensis*. *Plant Prot.* **2023**, *7*, 311–319.
39. Ahmad, G.; Khan, A.; Khan, A.A.; Ali, A.; Mohhamad, H.I. Biological control: A novel strategy for the control of the plant parasitic nematodes. *Anton. Leeuw. Int. J.* **2021**, *114*, 885–912. [CrossRef]
40. Sathyan, T.; Dhanya, M.K.; Murugan, M.; Ashokkumar, K.; Aswathy, T.S.; Narayana, R.; Deepthy, K.B. Evaluation of bio-agents, synthetic insecticides and organic amendment against the root-knot nematode, *Meloidogyne* spp. in cardamom [*Elettaria cardamomum* (L.) Maton]. *J. Biol. Control* **2021**, *35*, 68–75.
41. Alves, L.E.S.G.; Fontana, L.F.; Dias-Arieira, C.R. Green manure and *Pochonia chlamydosporia* for *Meloidogyne javanica* control in soybean 1. *Rev. Caatinga* **2022**, *35*, 625–632. [CrossRef]
42. Krif, G.; Lahlali, R.; El Aissami, A.; Laasli, S.E.; Mimouni, A.; Serderidis, S.; Picaud, T.; Moens, A.; Dababat, A.A.; Fahad, K.; et al. Efficacy of authentic bio-nematicides against the root-knot nematode, *Meloidogyne javanica* infecting tomato under greenhouse conditions. *Physiol. Mol. Plant Pathol.* **2022**, *118*, 101803. [CrossRef]
43. Poveda, J.; Baptista, P. Filamentous fungi as biocontrol agents in olive (*Olea europaea* L.) diseases: Mycorrhizal and endophytic fungi. *Crop Prot.* **2021**, *146*, 105672. [CrossRef]
44. Bo, T.; Kong, C.; Zou, S.; Mo, M.; Liu, Y. *Bacillus nematocida* B16 enhanced the rhizosphere colonization of *Pochonia chlamydosporia* ZK7 and controlled the efficacy of the root-knot nematode *Meloidogyne incognita*. *Microorganisms* **2022**, *10*, 218. [CrossRef]
45. Carvalho, R.P.; Vieira dos Santos, M.C.; Almeida, M.T.M.; Costa, S.R. Effects of commercial pesticides on the nematode biological control agent *Pochonia chlamydosporia*. *Biocontrol Sci. Technol.* **2022**, *32*, 1220–1231. [CrossRef]

46. Das, N.; Waquar, T. Bio-Efficacy of *Purpureocillium lilacinum* on management of root-knot nematode, *Meloidogyne incognita* in tomato. *Ind. J. Nematol.* **2021**, *51*, 129–136. [CrossRef]
47. Giannakou, I.O.; Tasoula, V.; Tsafara, P.; Varimpopi, M.; Antoniou, P. Efficacy of *Purpureocillium lilacinum* in combination with chitosan for the control of *Meloidogyne javanica*. *Biocontrol Sci. Technol.* **2020**, *30*, 671–684. [CrossRef]
48. Zhang, X.; Song, M.; Li, J.; Liu, X.; Gao, L.; Tian, Y. Changes in Soil Nematode and Microbial Community in Cucumber Root-Zone Soil Shaped by Intercropping with Amaranth. *Horticulturae* **2023**, *9*, 924. [CrossRef]
49. Watson, T.T.; Nelson, L.M.; Neilsen, D.; Neilsen, G.H.; Forge, T.A. Soil amendments influence *Pratylenchus penetrans* populations, beneficial rhizosphere microorganisms, and growth of newly planted sweet cherry. *Appl. Soil Ecol.* **2017**, *117*, 212–220. [CrossRef]
50. Bawa, N.; Kaur, S.; Dhillon, N.K. Efficacy of *Purpureocillium lilacinum*, *Trichoderma harzianum* and *T. viride* bio-formulations against *Meloidogyne incognita*. *Indian Phytopathol.* **2020**, *73*, 799–804. [CrossRef]
51. Kaur, K.; Thakur, N.; Sharma, S. Biological control of plant parasitic nematodes associated with capsicum (*Capsicum annuum* L.). *Ind. J. Nematol.* **2020**, *50*, 1–6.
52. Arita, L.Y.; da Silva, S.A.; Machado, A.C.Z. Efficacy of chemical and biological nematicides in the management of *Meloidogyne paranaensis* in *Coffea arabica*. *Crop Prot.* **2020**, *131*, 105099. [CrossRef]
53. Morgan-Jones, G.; White, J.; Rodriguez-Kabana, R. Phytonematode pathology: Ultrastructural studies. II. Parasitism of *Meloidogyne arenaria* eggs and larvae by *Paecilomyces lilacinus*. *Nematropica* **1984**, *14*, 57–71.
54. Holland, R.; Williams, K.; Khan, A. Infection of *Meloidogyne javanica* by *Paecilomyces lilacinus*. *Nematology* **1999**, *1*, 131–139. [CrossRef]
55. Cayrol, J.C.; Djian, C.; Pijarowski, L. Study of the nematicidal properties of the culture filtrate of the nematophagous fungus *Paecilomyces lilacinus*. *Rev. De Nematol.* **1989**, *12*, 331–336.
56. Khan, M.; Goswami, B. Effect of culture filtrates of *Paecilomyces lilacinus* isolates on hatching of *Meloidogyne incognita* eggs. *Ann. Plant Pro. Sci.* **2000**, *8*, 62–65.
57. Isogai, A.; Suzuki, A.; Higashkawa, S.; Kuyama, S.; Tamura, S. Constituents of a peptidal antibiotic P168 produced by *Paecilomyces lilacinus* (Thom) Samson. *Agric. Biol. Chem.* **1980**, *44*, 3029–3031. [CrossRef]
58. Freire, F.; Bridge, J. Parasitism of eggs, females and juveniles of *Meloidogyne incognita* by *Paecilomyces lilacinus* and *Verticillium chlamydosporium*. *Fitopatol. Brasil.* **1985**, *10*, 577–596.
59. Lopez-Llorca, L.; Duncan, G. A study of fungal endoparasitism of the cereal cyst nematode (*Heterodera avenae*) by scanning electron microscopy. *Can. J. Microbiol.* **1988**, *34*, 613–619. [CrossRef]
60. Lopez-Llorca, L.; Claugher, D. Appressoria of the nematophagous fungus *Verticillium suchlasporium*. *Micron. Microsc. Acta* **1990**, *21*, 125–130. [CrossRef]
61. Morton, O.; Hirsch, P.; Kerry, B. Infection of plant-parasitic nematodes by nematophagous fungi–A review of the application of molecular biology to understand infection processes and to improve biological control. *Nematology* **2004**, *6*, 161–170. [CrossRef]
62. Lopez-Llorca, L.V.; Robertson, W.M. Immunocytochemical localization of a 32-kDa protease from the nematophagous fungus *Verticillium suchlasporium* in infected nematode eggs. *Exp. Mycol.* **1992**, *16*, 261–267. [CrossRef]
63. Mukhtar, T.; Hussain, M.A.; Kayani, M.Z. Biocontrol potential of *Pasteuria penetrans*, *Pochonia chlamydosporia*, *Paecilomyces lilacinus* and *Trichoderma harzianum* against *Meloidogyne incognita* in okra. *Phytopathol. Mediterr.* **2013**, *52*, 66–76.
64. Kerry, B.R. Exploitation of the nematophagous fungus *Verticillium chlamydosporium* Goddard for the biological control of root-knot nematodes (*Meloidogyne* spp.). In *Fungi as Biocontrol Agents: Progress, Problems and Potential*; CAB International: Wallingford, UK, 2001; pp. 155–168.
65. Sharon, E.; Chet, I.; Viterbo, A.; Bar-Eyal, M.; Nagan, H.; Samuels, G.J.; Spiegel, Y. Parasitism of *Trichoderma* on *Meloidogyne javanica* and role of the gelatinous matrix. *Eur. J. Plant Pathol.* **2007**, *118*, 247–258. [CrossRef]
66. Affokpon, A.; Coyne, D.L.; Htay, C.C.; Agbèdè, R.D.; Lawouin, L.; Coosemans, J. Biocontrol potential of native *Trichoderma* isolates against root-knot nematodes in West African vegetable production systems. *Soil Biol. Biochem.* **2011**, *43*, 600–608. [CrossRef]
67. Saikia, S.K.; Tiwari, S.; Pandey, R. Rhizospheric biological weapons for growth enhancement and *Meloidogyne incognita* management in *Withania somnifera* cv. Poshita. *Bio. Control* **2013**, *65*, 225–234. [CrossRef]
68. Sonkar, S.S.; Bhatt, J.; Meher, J.; Kashyap, P. Bio-efficacy of *Trichoderma viride* against the root-knot nematode (*Meloidogyne incognita*) in tomato plant. *J. Pharmacogn. Phytochem.* **2018**, *7*, 2010–2014.
69. Sharon, E.; Chet, I.; Spiegel, Y. Improved attachment and parasitism of *Trichoderma* on *Meloidogyne javanica* in vitro. *Eur. J. Plant Pathol.* **2009**, *123*, 291–299. [CrossRef]
70. Ragozzino, A.; d'Errico, G. Interactions between nematodes and fungi: A concise review. *Redia* **2012**, *94*, 123–125.
71. Lombardi, N.; Vitale, S.; Turrà, D.; Reverberi, M.; Fanelli, C.; Vinale, F.; Lorito, M. Root exudates of stressed plants stimulate and attract *Trichoderma* soil fungi. *Mol. Plant-Microbe Inter.* **2018**, *31*, 982–994. [CrossRef]
72. Suarez, B.; Rey, M.; Castillo, P.; Monte, E.; Llobell, A. Isolation and characterization of PRA1, a trypsin-like protease from the biocontrol agent *Trichoderma harzianum* CECT 2413 displaying nematicidal activity. *App. Microbiol. Biotechnol.* **2004**, *65*, 46–55. [CrossRef]
73. Yang, Z.S.; Li, G.H.; Zhao, P.J.; Zheng, X.; Luo, S.L.; Li, L.; Zhang, K.Q. Nematicidal activity of *Trichoderma* spp. and isolation of an active compound. *World J. Microbiol. Biotechnol.* **2010**, *26*, 2297–2302. [CrossRef]
74. Yang, Z.; Yu, Z.; Lei, L.; Xia, Z.; Shao, L.; Zhang, K.; Li, G. Nematicidal effect of volatiles produced by *Trichoderma* sp. *J. Asia-Pac. Entomol.* **2012**, *15*, 647–650. [CrossRef]

75. Spiegel, Y.; Sharon, E.; Chet, I. Mechanisms and improved biocontrol of the root-knot nematodes by *Trichoderma* spp. *Acta Hort.* **2005**, *698*, 225–228. [CrossRef]
76. Bokhari, F.M. Efficacy of Trichoderma species in the control of *Rotylenchulus reniformis* and *Meloidogyne javanica*. *Arch. Phytopathol. Plant Prot.* **2009**, *42*, 361–369. [CrossRef]