Integrated management of plant-parasitic nematodes on guava and fig trees under tropical field conditions

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Abstract

Two field experiments were carried out to study the efficacy of different biological control agents in controlling certain plant-parasitic nematode species including Meloidogyne javanica, Tylenchorhynchus medierraneus, Hoplolaimus seinhorsti, Longidorus latocephalus, and Xiphinema elongatum on guava and fig trees under the tropical field conditions of Jazan region, south-west Saudi Arabia during two successive seasons from Feb. 15, 2016 to Jan. 15, 2017. The evaluated bio-agents were used in different integrated management combinations of certain fungal species (Trichoderma harzianum, Verticillium chlamydosporium, and Purpureocillium lilacinum), the bacterium Pasteuria penetrans, some organic amendments (cow manure, compost, and chicken manure), urea 46% as a nitrogenous fertilizer, and the nematicide carbofuran 10G for comparison. Results showed that all the tested treatments gradually decreased (P ≤ 0.05) the population densities of plant-parasitic nematodes on guava and fig trees over the study period. The highest reduction of nematode densities occurred at the end of the experiment. Carbofuran 10G was the most effective treatment in suppressing the nematode densities on guava and fig trees. The most effective management combinations, next to carbofuran 10G, in suppressing the nematode densities in the rhizosphere of guava trees were P. lilacinum + P. penetrans + urea 46%, P. lilacinum + P. penetrans + chicken manure, and T. harzianum + P. penetrans + chicken manure (66.54–69.22% nematode reductions). Correspondent combinations in the rhizosphere of fig trees were P. lilacinum + P. penetrans + cow manure, T. harzianum + P. penetrans + cow manure, P. lilacinum + P. penetrans + urea 46%, and V. chlamydosporium + P. penetrans + urea 46% (54.68–57.17% nematode reductions). On the other hand, nematode population densities continued to increase (P ≤ 0.05) in the rhizosphere of guava and fig trees in the absence of nematode management combinations. All the tested treatments significantly increased (P ≤ 0.05) the number of fruits/tree on guava and fig trees. Treatments which included the combinations of fungal and bacterial parasites along with chicken manure gave the highest numbers of fruits/tree, followed by the treatment with the nematicide carbofuran 10G. Regression analysis showed a significant negative linear relationship between the number of nematodes/kg soil and the number of guava and fig fruits/tree.

Keywords: Plant-parasitic nematodes, Integrated management, Ficus carica, Psidium guajava, Saudi Arabia

Background

Jazan region, southwest corner of Saudi Arabia, is noted for its high-quality production of some tropical and subtropical fruits including mango, fig, guava, banana, papaya, and avocado (Basha 1998). In the past two decades, the root-knot nematodes, Meloidogyne spp. Göldi, have been reported on some species of the tropical fruit trees grown in the region, especially guava, Psidium guajava L. and fig, Ficus carica L. (Mokbel 2014). Gomes et al. (2008) showed that guava trees infected, with Meloidogyne mayaguensis Rammah & Hirschmann, suffered from the deficiency of nitrogen, potassium, phosphorus, calcium, and magnesium, and these mineral deficiencies were proportionally related to the severity of root galling and root decay, which ultimately led to the death of guava trees within a few months. Unlike, the persistence of chemical nematicides is usually short in the tropics, due to run-off and leaching. Besides, many of the effective nematicides were banned from the markets owing to their risks to human
health and environment. So, alternative control measures should be adopted to replace those compounds. The concept of combining compatible tactics for controlling nematodes predates that of integrated pest management (IPM) (Barker 2013).

The biological control agents of nematodes include many microorganisms, but the most important are fungi and bacteria. *Pseudomonas fluorescens* Migula were combined to enrich the farm yard manure, which was added to the rhizosphere of papaya seedlings, the root populations of many pathogenic nematode species (Jatala 1986). For example, it effectively controlled *Tylenchulus semipenetrans* Cobb on mandarin and rough lemon, and *Linford & Oliveira* and *Rotylenchulus reniformis* Migula were announced to be the most potent fungal parasites that can effectively control *Meloidogyne* spp. on many host plants (Rao 2007). *Pasteuria penetrans* Sayre & Starr, a mycelial endospore-forming bacterial parasite, represents another successful bio-control agent against root-knot nematodes (Chen et al. 1996). *P. lilacinum* offers a successful biological control against many pathogenic nematode species (Jatala 1986). As well, it effectively controlled *Tylenchulus semipenetrans* Cobb on mandarin and rough lemon, and the results were best when the fungus was combined with oil-cakes (Le Roux et al. 2000). Also, when *P. lilacinum* and the bacteria *Pseudomonas fluorescens* Migula were combined to enrich the farm yard manure, which was added to the rhizosphere of papaya seedlings, the root populations of *Rotylenchulus reniformis* Linford & Oliveira and *Meloidogyne incognita* (Kofoid & White) Chitwood were reduced by 73% and 78%, respectively, and the papaya yield was increased by 26% (Rao 2010). As well, *T. harzianum* effectively suppressed the population of the root-knot nematode, *M. enterolobii* Yang & Eisenback in both soil and roots of guava in Thailand (Jindapunnapat et al. 2013). When the nursery soil of papaya trees was treated by *T. harzianum* and the rhizobacteria *P. fluorescens*, either in a single or combined applications, *M. incognita* was greatly controlled and the papaya yield was increased (Rao 2007). De Leij et al. (1992) reported the potential of some *Verticillium chlamydosporium* isolates against *M. arenaria* on tomato plants.

In Saudi Arabia, Al-Hazmi et al. (2013) found a heavy colonization of the cysts of *Heterodera avenae* Woll. with the fungus *Verticillium chlamydosporium*. The bacterium *P. penetrans* has shown a great control potential against many plant-parasitic nematode species, especially *Meloidogyne* spp. (Chen and Dickson 1998). This bacterial parasite has been reported adhering to, or infesting hundreds of nematode species from many countries worldwide (Sturhan 1988). Al-Rehiyani (2007) reported the potential of *P. penetrans* in controlling *M. incognita* on grape in Al-Qasim region, Saudi Arabia.

Organic and inorganic nitrogenous amendments, which have been usually added to soil to improve soil fertility, have also offered good nematicidal effects against plant-parasitic nematodes (Oka 2010). Urea and ammonia were found to be effective in controlling the plant-parasitic nematodes at rates as low as 300–400 mg/kg soil (Rodriguez-Kabana 1986). Guava decline disease, a complex disease involving *M. mayaguensis* and *Fusarium solani* Keratitidis, has been greatly managed in a commercial guava plantation and a major yield gains were obtained by the applications of cow manure and poultry compost (Gomes et al. 2010).

This study aimed to evaluate different bio-control agents in an integrated management combinations to manage the nematode problems on guava and fig trees under the tropical field conditions of Jazan region, southwest of Saudi Arabia.

**Materials and methods**

Two field experiments were carried out in a 2-year-old guava and fig orchards located at Abu Areesh governorate, Jazan region, southwest of Saudi Arabia to study the efficacy of different integrated combinations of biological control agents in controlling certain plant-parasitic nematode (PPN) species.

**Nematode infestation and identification**

Soil of guava and fig orchards were naturally infested with a group of plant-parasitic nematode (PPN) species including *Meloidogyne javanica* (Treub) Chitwood, *Tylenchorhynchus mediterraneus* Handoo, *Hoplolaimus seinhorsti* Luc, *Longidorus latocephalus* Lamberti, Choleva and Agostinelli, and *Xiphinema elongatum* Schuurmans Stekhoven & Teunissen. These nematode species were morphologically and molecularly characterized by Dawabah and Al-Yahya (2017), and their frequency of occurrence (FO %) in both guava and fig orchards at the beginning of the experiments were determined (Table 1). The experiments were carried out during two successive seasons from Feb. 15, 2016 to Jan. 15, 2017. Guava and fig trees were spaced (5 × 5 m), and irrigated by sprinklers as needed.

One week prior to the implementation of the two experiments on guava and fig trees (8th of Feb., 2016), rhizosphere soil samples were collected from under the trees of the two orchards, representing the experimental units for the different treatments, to extract and count the initial population densities (Pi) of PPN species in each soil sample.

### Table 1 Frequency of occurrence (FO%) of plant-parasitic nematodes associated with guava, *Psidium guajava* and fig, *Ficus carica*, at the beginning of the management experiments

| Genus/species               | Guava Frequency of occurrence (FO %) | Fig Frequency of occurrence (FO %) |
|-----------------------------|--------------------------------------|-----------------------------------|
| *Hoplolaimus seinhorsti*    | 24.1                                 | 13.0                              |
| *Longidorus latocephalus*   | 13.0                                 | 16.7                              |
| *Meloidogyne javanica*      | 57.4                                 | 53.7                              |
| *Tylenchorhynchus mediterraneus* | 46.3                              | 41.5                              |
| *Xiphinema elongatum*       | 5.0                                  | 8.9                               |

Frequency of occurrence (FO%) = (number of samples containing a species / number of collected samples) × 100
experimental unit (tree). Accordingly, trees with approximately close numbers of PPNs were selected, labeled, and assigned randomly at four replicates (trees)/treatment, in a complete randomized design (CRD) (Siddiqi et al. 2007). Beside, a map was designed for each experiment.

Identification and preparation of fungal and bacterial inocula

*T. harzianum* and *V. chlamydosporium* were isolated from the egg masses of the root-knot nematode, *M. javanica*, collected from galled guava roots grown in Riyadh region, central Saudi Arabia. Egg masses were surface sterilized by 0.1% NaOCl for 30 s, washed three times in a sterile distilled water, and then transferred to Petri dishes containing sterilized potato dextrose agar (PDA). Petri dishes were incubated at 25 °C and observed for fungal growth. Pure isolates of *T. harzianum* and *V. chlamydosporium*, isolated and identified, based on the cultural and spore morphological features, were also sent to the Plant Pathology Research Institute, Agricultural Research Center, Giza, Egypt to confirm the identification. An aggressive German isolate of *P. lilacinum* (DSMZ 14052) was obtained from Damascus University. The fungi: *T. harzianum*, *V. chlamy- dosporium*, and *P. lilacinum* were continuously maintained on PDA for further use.

To prepare fungal inocula, wheat grains were immersed in water over night, and weights of 250 g of wetted grains were transferred to 500 ml conical flasks, which were autoclaved at 15 psi for 20 min twice. Each flask containing sterilized wheat grains was inoculated by a couple of 2-mm discs of the designated fungus and incubated at 25 °C for 2 weeks. The flasks were shaken every 3–4 days to ensure the uniform colonization of the fungus. For fungal infestation in the field soil, the basin of each tree was infested with 1 kg of fungal-infected wheat grains, distributed in the top 20 cm soil of the tree basin.

A local isolate of the endospore forming bacteria, *P. penetrans*, was isolated from *M. javanica* second-stage juveniles (J2), parasitizing olive roots at Al-Melaidah, Al-Qasim, Saudi Arabia. This local bacterial isolate was previously identified on a molecular basis by Al-Rehiyani and Motawei (2014). *M. javanica* J2s, with bacterial endospores attached, were obtained by centrifugation (Hewlett and Dickson 1993), and used to inoculate susceptible tomato plants cv. “Sultana-7,” growing in a steam-sterilized sandy loam soil at 3000 J2s/plant/pot. Tomato plants were harvested, 60 days after inoculation, uprooted and the root systems were washed, then air dried on a lab. bench. Aliquots of root materials (0.5 g/5 ml water) were homogenized, using a pestle and a mortar. Homogenates were then passed through a 35 mesh (50 µ openings) sieve (Bird and Brisbane 1988). Number of released endospores in the suspension was determined, using a hemocytometer, and adjusted to a concentration of 1 × 10⁷/ml. The bacterial inoculum consisted of 20 ml of the bacterial endospore suspension/tree basin (Abd-Elgawad et al. 2010).

The nematicidal and organic amendments

Cow manure (organic matter = 50%, pH = 7.5, humidity = 20–25%, C/N ratio = 25:1) and compost (organic matter = 52%, pH = 7.0, humidity = 20%, C/N ratio = 30:1) were used at 300 kg/ha (1 kg/tree) (Siddiqi et al. 2007), while chicken manure (organic matter = 82.35%, pH = 7.0, humidi- ty = 21.6%, C/N ratio = 20:1) and urea (46% nitrogen) were added at 50 g/tree. All organic materials were ob- tained from the local market, as pure sterilized dry powders. For comparison, the nematicide, carbofuran 10G was used at 40 g/tree. All the tested materials were incorpo- rated uniformly in the top 20 cm soil of the tree basin.

Application of treatments

The first application of the treatments took place on the 15th of Feb., 2016. Three months later (on the 15th of May, 2016), root and rhizosphere soil samples were collected from the trees of both experiments to determine the numbers of nematodes/tree, and the mean number of nematodes/treatment. The treatments (T1–T14) included:

- T1: *P. lilacinum* + *P. penetrans* + chicken manure.
- T2: *V. chlamydosporium* + *P. penetrans* + chicken manure.
- T3: *T. harzianum* + *P. penetrans* + chicken manure.
- T4: *P. lilacinum* + *P. penetrans* + cow manure.
- T5: *V. chlamydosporium* + *P. penetrans* + cow manure.
- T6: *T. harzianum* + *P. penetrans* + cow manure.
- T7: *P. lilacinum* + *P. penetrans* + urea 46%.
- T8: *V. chlamydosporium* + *P. penetrans* + urea 46%.
- T9: *T. harzianum* + *P. penetrans* + urea 46%.
- T10: *P. lilacinum* + *P. penetrans* + compost.
- T11: *V. chlamydosporium* + *P. penetrans* + compost.
- T12: *T. harzianum* + *P. penetrans* + compost.
- T13: Carbofuran 10 G
- T14: Non-treated trees (check).

The second application of the treatments (same as in the first one) took place on the 15th of Oct., 2016, and also number of nematodes per each tree was determined. Three months later (on the 15th of Jan., 2017), rhizosphere soil samples were collected from all the experimental units (trees) and the mean number of nematodes/treatment (final nematode populations = Pf) was determined in both experiments.

Data analysis

Data were statistically analyzed, using SPSS (2016), and means were separated using Fisher’s Protected LSDₐ₀.₀₅.
**Results and discussion**

All the tested treatments significantly reduced ($P < 0.05$) the population densities of PPN in the rhizosphere of guava and fig trees in the two separate field experiments (Tables 2 and 3). Nematode populations (M. javanica, T. mediterraneus, H. seinhorsii, L. latocephalus, and X. elongatum) gradually decreased at all the tested treatments over the study period from Feb. 15, 2016 to Jan. 15, 2017. The highest reductions were achieved by the end of the experiments (final nematode populations) (Tables 2 and 3). In both experiments, carbofuran 10G was the most effective treatment in suppressing the nematode population densities in the rhizosphere of guava and fig trees (80.13 and 83.13%, respectively). Actually, chemical treatments mostly have the advantage of the quick and effective response in controlling plant-parasitic nematodes. Soltani et al. (2013) found that aldicarb, enzone, oxamyl, and cadusafos at 6 and 8 ppm concentrations were effective treatments in controlling the root-knot nematode, M. javanica, on 1-year old olive seedlings in the greenhouse.

Next to carbofuran treatment, the most effective combinations of the tested treatments in suppressing the nematode population densities in the rhizosphere of guava trees were $P. lilacinum + P. penetrans + urea 46\%$, $P. lilacinum + P. penetrans + chicken manure$, and $T. harzianum + P. penetrans + chicken manure$ (66.54–69.22% nematode reductions), followed by $T. harzianum + P. penetrans + chicken manure$, $V. chlamydosporium + P. penetrans + urea 46\%$, and $T. harzianum + P. penetrans + urea 46\%$ (55.52–59.38% nematode reductions) (Table 2). Correspondent combinations in the rhizosphere of fig trees were $P. lilacinum + P. penetrans + cow manure$, $T. harzianum + P. penetrans + cow manure$, $P. lilacinum + P. penetrans + urea 46\%$, and $V. chlamydosporium + P. penetrans + urea 46\%$ (54.68–57.17% nematode reductions), followed by $V. chlamydosporium + P. penetrans + compost and $P. lilacinum + P. penetrans + chicken manure$ (39.27–49% nematode reductions) (Table 3).

On the other hand, nematode population densities gradually increased ($P < 0.05$) in the rhizosphere of guava and fig trees in the absence of nematode-control measures (non-treated control). These increments reached up to 75.3 and 35.46% in the rhizosphere of guava and fig trees, respectively, by the end of the experiments (Tables 2 and 3).

The ability of the different non-chemical combinations used in this study is greatly consistent with the results of previous studies. Many alternative control measures have been recently adopted to replace the chemical

**Table 2.** Effect of different combinations of integrated control measures on the number of plant-parasitic nematodes (PPN) associated with guava, *Psidium guajava*, rhizosphere

| Treatment                                      | Number of PPN/200 cm$^3$ soil | 15/2/2016$^a$ | 15/5/2016 | 15/10/2016$^b$ | 15/1/2017 | % final Change$^c$ |
|-----------------------------------------------|--------------------------------|---------------|------------|----------------|------------|------------------|
| $P. lilacinum + P. penetrans + chicken manure$ | 652.50 a                       | 492.50 b      | 348.75 c   | 202.50 d       | –68.97    |
| $V. chlamydosporium + P. penetrans + chicken manure$ | 640.00 a                       | 556.25 ab     | 486.25 b   | 260.00 c       | –59.38    |
| $T. harzianum + P. penetrans + chicken manure$ | 650.00 a                       | 511.25 b      | 450.00 b   | 217.50 c       | –66.54    |
| $P. lilacinum + P. penetrans + cow manure$    | 717.50 a                       | 662.50 a      | 537.50 b   | 380.00 c       | –47.04    |
| $V. chlamydosporium + P. penetrans + cow manure$ | 851.25 a                       | 772.50 a      | 793.75 a   | 488.75 b       | –36.73    |
| $T. harzianum + P. penetrans + cow manure$    | 757.50 a                       | 736.25 ab     | 651.25 b   | 433.75 c       | –42.74    |
| $P. lilacinum + P. penetrans + compost$       | 732.50 a                       | 562.50 b      | 484.00 b   | 396.25 c       | –45.90    |
| $V. chlamydosporium + P. penetrans + compost$ | 741.25 a                       | 788.75 a      | 590.00 b   | 567.50 b       | –23.44    |
| $T. harzianum + P. penetrans + compost$       | 715.00 a                       | 651.25 ab     | 581.50 bc  | 536.25 c       | –17.66    |
| $P. lilacinum + P. penetrans + urea 46\%$     | 683.75 a                       | 608.75 b      | 512.50 b   | 210.00 c       | –69.29    |
| $V. chlamydosporium + P. penetrans + urea 46\%$ | 736.25 a                       | 633.75 b      | 553.75 b   | 327.50 c       | –55.52    |
| $T. harzianum + P. penetrans + urea 46\%$     | 755.00 a                       | 661.25 b      | 642.50 b   | 328.75 c       | –56.46    |
| Carbofuran 10G                                | 773.75 a                       | 327.50 b      | 175.00 c   | 153.75 c       | –80.13    |
| Nematode control                              | 738.75 d                       | 993.75 c      | 1170.00 b  | 1295.00 a      | +75.30    |

* LSD$_{0.05}$ between treatments

| LSD$_{0.05}$ between times | 46.98 |

| LSD$_{0.05}$ between treatments $\times$ times | 175.77 |

Data are average of four replicates (trees) each

Values followed by the same letter(s) in a row are not significantly different at $P < 0.05$

* $P. lilacinum =$ *Purpureocillium lilacinum*, $P. penetrans =$ *Pasteuria penetrans*, $V. chlamydosporium =$ *Verticillium chlamydosporium*, $T. harzianum =$ *Trichoderma harzianum*

$^a$First treatments application

$^b$Second treatments application (beginning of the second season)

$^c$Compared to the number of PPN at the beginning of the experiment (Pi)
Table 3 Effect of different combinations of integrated control measures on the number of plant-parasitic nematodes (PPN) associated with fig, Ficus carica, rhizosphere

| Treatment | Number of PPN/200 cm$^3$ soil | 15/2/2016$^a$ | 15/5/2016 | 15/10/2016$^b$ | 15/1/2017 | % final Change$^c$ |
|-----------|---------------------------------|---------------|------------|---------------|------------|-------------------|
| P. lilacinum + P. penetrans + chicken manure | 547.50 a | 483.75 ab | 457.50 b | 332.50 c | 332.50 c | -39.27 |
| V. chlamydosporium + P. penetrans + chicken manure | 507.50 a | 400.00 b | 440.00 ab | 393.75 b | 393.75 b | -22.41 |
| T. harzianum + P. penetrans + chicken manure | 557.50 a | 532.50 ab | 472.50 bc | 383.75 c | 383.75 c | -31.17 |
| P. lilacinum + P. penetrans + cow manure | 641.25 a | 612.50 a | 327.50 b | 288.75 b | 288.75 b | -54.97 |
| V. chlamydosporium + P. penetrans + cow manure | 535.00 a | 448.75 c | 545.00 b | 403.75 c | 403.75 c | -24.53 |
| T. harzianum + P. penetrans + cow manure | 516.25 a | 438.75 b | 292.50 c | 223.75 c | 223.75 c | -56.66 |
| P. lilacinum + P. penetrans + compost | 632.50 a | 533.75 ab | 498.75 b | 387.50 c | 387.50 c | -38.74 |
| V. chlamydosporium + P. penetrans + compost | 625.00 a | 615.00 a | 522.50 b | 318.75 c | 318.75 c | -49.00 |
| T. harzianum + P. penetrans + compost | 536.25 a | 422.50 b | 405.00 b | 302.50 c | 302.50 c | -43.59 |
| P. lilacinum + P. penetrans + urea 46% | 575.00 a | 377.50 b | 330.00 bc | 246.25 c | 246.25 c | -57.17 |
| V. chlamydosporium + P. penetrans + urea 46% | 695.00 a | 531.25 b | 497.50 b | 315.00 c | 315.00 c | -54.68 |
| T. harzianum + P. penetrans + urea 46% | 568.75 a | 458.75 b | 420.00 bc | 360.00 c | 360.00 c | -36.70 |
| Carbofuran10G | 622.50 a | 115.00 b | 160.00 b | 105.00 b | 105.00 b | -83.13 |
| Nematode control | 606.25 c | 712.50 b | 781.25 ab | 821.25 a | 821.25 a | + 35.46 |

Data are average of four replicates (trees) each
Values followed by the same letter(s) in a row are not significantly different at $P \leq 0.05$
$P. lilacinum =$ Purpureocillium lilacinum, $P. penetrans =$ Pasteuria penetrans, $V. chlamydosporium =$ Verticillium chlamydosporium, $T. harzianum =$ Trichoderma harzianum
$^a$First treatments application
$^b$Second treatments application (beginning of the second season)
$^c$Compared to the number of PPN at the beginning of the experiment (Pi)

As shown, in the present study, reductions of the nematode densities in the rhizosphere of guava and fig trees were much higher when chicken manures, cow manure, and urea 46% were added along with the fungal and bacterial parasites. These findings are in agreement with the results of previous studies which used the fungus $P. lilacinum$ and the bacteria $P. fluorescens$ to enrich the farm yard manure, then applied the enriched manure to the rhizosphere of papaya seedlings to effectively control the reniform nematode, $R. reniformis$, and the root-knot nematode, $M. incognita$ (Rao 2007 and 2010). Similarly, the fungus $P. lilacinum$ was previously found suppressing the population densities of the citrus nematode, $T. semipenetrans$, on citrus seedlings in pot experiments, and the results were best when the fungus was combined with organic amendments (oil-cakes) (Le Roux et al. 2000). Gomes et al. (2010) concluded also that coupling the control treatments with organic soil amendments, particularly poultry compost and cow manure spread evenly under the guava canopy, gave a better control of the root-knot nematode, $M. mayaguensis$. In fact, organic soil amendments stimulate the activities of soil microorganisms that are antagonistic to plant-parasitic nematodes. The decomposition of organic matter results in the accumulation of specific compounds in the soil that may have nematicidal effects against nematodes (Akhtar and Malik 2000). In addition, long-term effects might include increases in the population densities of the nematode antagonists in the soil. Also, improved crop nutrition and plant...
growth following amendments use might lead to tolerance of plants against plant-parasitic nematodes (McSorley 2011). However, urea is readily converting to the toxic ammonia (NH₃) by the urease enzyme, which is readily present in the soil (Rodriguez-Kabana 1986). The nematicidal properties of ammonia could be attributed to either its plasmolyzing effect in the immediate vicinity of its application site in the soil, or the possibility that ammonia could exert a selective influence for microbial antagonists of nematodes, particularly fungi (Rodriguez-Kabana 1986; Chavarria-Carvajal and Rodriguez-Kabana 1998 and Santana-Gomes et al. 2013).

Both guava and fig have two fruiting periods a year in Jazan region, southwest of Saudi Arabia (Mars to May and November to January of the second year). In both periods during the present study, all the tested treatments significantly increased \((P \leq 0.05)\) the number of fruits/tree either for guava or fig (Tables 4 and 5). Treatments which included the combinations between fungal and bacterial parasites along with chicken manure had the highest numbers of guava fruits/tree, followed by the treatment with the nematicide carbofuran 10G (Table 4). However, treatments of fungal and bacterial parasites enriched with any of the chicken manure, urea 46%, cow manure, or compost also gave the highest numbers of fig fruits/tree like the nematicide, carbofuran 10G or may be more (Table 5). These findings might be greatly supported with some previous studies, which have repeatedly proved the effectiveness of carbamate and organophosphorus nematicides in controlling plant-parasitic nematodes and increasing the yield of some tropical and subtropical fruit trees in different countries (Quenejerve et al. 1991). Similarly, other previous studies also proved the usefulness of some non-chemical treatments such as cow manure and poultry compost in managing the plant parasitic nematodes attacking guava trees and increasing their fruit yields (Souza et al. 2006 and Gomes et al. 2010). Obtained results also obviated that adding each of urea 46%, chicken manure, or cow manure to the tested fungal parasites and the bacteria \(P. \) penetrans increased the yield of guava and fig trees, and these yield increments were sometimes higher than those of increments gained by the use of carbofuran 10G. These results are consistent with the findings of Rao (2010) who reported that the fungus \(P. \) lilacinum and the bacteria \(P. \) fluorescens enriched the farm yard manure fairly controlled the reniform nematode, \(R. \) reniformis and the root-knot

| Table 4 Effect of different combinations of integrated control measures on the number of guava, Psidium guajava, fruits |
|---------------------------------------------------------------|
| **Treatment** | **Number of fruits/tree** | **1st yield** | **2nd yield** |
|----------------|-----------------------------|---------------|---------------|
| \(P. \) lilacinum + \(P. \) penetrans + chicken manure | 86.75 a | 64.25 a | 45.50 c |
| \(V. \) chlamydosporium + \(P. \) penetrans + chicken manure | 81.00 b | 45.25 d | 36.25 d |
| \(T. \) harzianum + \(P. \) penetrans + chicken manure | 53.25 c | 27.50 f | 25.00 gh |
| \(V. \) chlamydosporium + \(P. \) penetrans + cow manure | 27.50 f | 21.75 gh | 45.00 c |
| \(T. \) harzianum + \(P. \) penetrans + cow manure | 22.50 ghi | 21.75 gh | 45.00 c |
| \(V. \) chlamydosporium + \(P. \) penetrans + compost | 23.50 ghi | 22.50 ghi | 45.00 c |
| \(V. \) chlamydosporium + \(P. \) penetrans + compost | 20.25 e | 20.25 e | 45.00 c |
| \(T. \) harzianum + \(P. \) penetrans + compost | 20.50 e | 35.75 de | 35.75 de |
| \(P. \) lilacinum + \(P. \) penetrans + urea 46% | 50.25 e | 22.00 i | 22.00 i |
| \(V. \) chlamydosporium + \(P. \) penetrans + urea 46% | 44.50 e | 45.75 e | 29.25 f |
| \(T. \) harzianum + \(P. \) penetrans + urea 46% | 45.75 e | 70.25 c | 52.75 b |
| Carbofuran 10G | 16.75 j | 16.75 j | 19.50 j |
| Nematode control | 2.44 | 2.44 | 2.44 |
| LSD\(_{0.05}\) between treatments | 2.35 | 2.35 | 2.35 |
| LSD\(_{0.05}\) between time | 8.82 | 8.82 | 8.82 |

Data are average of four replicates (trees) each
Values followed by the same letter(s) in a column are not significantly different at \(P \leq 0.05\)

\(P. \) lilacinum = Purpureocillium lilacinum, \(P. \) penetrans = Pasteuria penetrans, \(V. \) chlamydosporium = Verticillium chlamydosporium, \(T. \) harzianum = Trichoderma harzianum
nematode, *M. incognita* and increased the yield of papaya crop by 26%.

Regression analysis showed a significant negative linear relationship between the number of nematodes/kg soil and both the number of guava fruits/tree ($y = -0.03x + 54.54$) (Fig. 1) and fig fruits/tree ($y = -0.124x + 112.03$) (Fig. 2). It means that, in both relations, the number of fruits/tree gradually decreased as the number of nematodes/kg soil was increased. Similar results were previously obtained by Ibrahim (2002) and Kim and Ferris (2002).

**Conclusion**

It is concluded from the present study that carbofuran 10G was the most effective treatment in suppressing the nematode densities in the rhizosphere of guava and fig trees, followed by the combinations of the fungal (*P. lilacinum*, *T. harzianum*, and *V. chlamydosporium*) and

| Table 5 Effect of different combinations of integrated control measures on the number of fig, *Ficus carica*, fruits |
|---------------------------------|---------------------------------|---------------------------------|
| Treatment                        | 1st yield                      | 2nd yield                      |
|                                 | Mars–May 2016                  | Nov. 2016–Jan. 2017            |
| *P. lilacinum* + *P. penetrans* + chicken manure | 38.25 abc                      | 100.50 bcd                     |
| *V. chlamydosporium* + *P. penetrans* + chicken manure | 32.50 bcde                     | 97.75 bcd                      |
| *T. harzianum* + *P. penetrans* + chicken manure | 29.00 cde                      | 95.25 cd                       |
| *P. lilacinum* + *P. penetrans* + cow manure | 27.00 e                        | 91.75 de                       |
| *V. chlamydosporium* + *P. penetrans* + cow manure | 31.25 bcde                     | 81.75 fg                       |
| *T. harzianum* + *P. penetrans* + cow manure | 27.25 e                        | 63.75 h                        |
| *P. lilacinum* + *P. penetrans* + compost | 43.50 a                        | 82.25 ef                       |
| *V. chlamydosporium* + *P. penetrans* + compost | 28.25 de                      | 128.50 a                       |
| *T. harzianum* + *P. penetrans* + compost | 28.75 cde                      | 84.75 ef                       |
| *P. lilacinum* + *P. penetrans* + urea 46% | 33.25 bcde                     | 106.75 b                       |
| *V. chlamydosporium* + *P. penetrans* + urea 46% | 38.00 abcd                    | 78.75 g                        |
| *T. harzianum* + *P. penetrans* + urea 46% | 36.50 abcde                    | 98.75 bcd                      |
| Carbofuran 10G                       | 40.50 ab                       | 103.25 bc                      |
| Nematode control                      | 16.50 f                        | 35.75 i                        |
| LSD$_{0.05}$ between treatments       | 10.05                          |                                |
| LSD$_{0.05}$ between time             | 3.80                           |                                |
| LSD$_{0.05}$ between treatments × time | 14.21                         |                                |

Data are average of four replicates (trees) each

Values followed by the same letter(s) in a column are not significantly different at *P* ≤ 0.05

*P. lilacinum* = *Purpureocillium lilacinum*, *P. penetrans* = *Pasteuria penetrans*, *V. chlamydosporium* = *Verticillium chlamydosporium*, *T. harzianum* = *Trichoderma harzianum*

![Fig. 1](image-url)  
**Fig. 1** Relationship between the number of nematodes/kg soil and the number of guava fruits/tree
bacterial \((P.\ penetrans)\) parasites along with chicken and cow manures and urea 46\%. However, treatments which included the combinations of fungal and bacterial parasites along with chicken manure gave the highest numbers of guava and fig fruits/tree, followed by carbofuran 10G. The use of such effective bio-control combinations in managing plant-parasitic nematodes in the tropical guava and fig orchards should be encouraged due to the dangerous hazards of chemical nematicides.

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Availability of data and materials
The data sets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors’ contributions
AD participated in the planning and designing the experiments, implementation the experiments, nematode identification, analyzing data, and writing the manuscript. FA participated in the planning and implementation of the experiments, sampling, lab work, and writing the draft. HL participated in the implementation of the experiments, sampling, lab work, and collecting data. All authors read and approved the final manuscript.

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Ethics approval and consent to participate
The study was conducted on nematode species that are abundant in the ecosystem and does not require ethical approval. Approval of participation is ensured by all authors.

Consent for publication
The manuscript has not been published in completely or in part elsewhere.

Competing interests
The authors declare that they have no competing interests.

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