International Journal of Agriculture Sciences
ISSN: 0975-3710 & E-ISSN: 0975-9107, Volume 10, Issue 20, 2018, pp.-7415-7420.
Available online at https://www.bioinfopublication.org/jouarchive.php?opt=&jouid=BPJ0000217

Research Article

ECOFRIENDLY MANAGEMENT OF WILT COMPLEX IN BLACK PEPPER (*Piper nigrum* L.)

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Received: October 14, 2018; Revised: October 25, 2018; Accepted: October 26, 2018; Published: October 30, 2018

Abstract: Black pepper (*Piper nigrum* L.), known as the "King of spices" and "black gold" is prone to attack by burrowing nematode, *Radopholus similis* and fungal wilt pathogen, *Phytophthora capsici* causing wilt complex and considerable yield loss. The field experiment was carried out in the farmer's field, with a view to evaluate bioagents (*Trichoderma harzianum*, *Purpureomyces lilacinum*, *Pseudomonas fluorescens* and *Bacillus subtilis*), organic amendment (Neem cake) and chemicals (Carbofuran and Bordeaux mixture) separately and in combination for the management of *Radopholus similis* and *Phytophthora capsici*. The final population of *R. similis* in soil was lowest in T4: Bordeaux mixture (1 %) + *P. lilacinum* (50 g) (302.66/200 cc soil), followed by T6: Carbofuran 3G (15 g) (335.33 nematodes/200 cc soil), T13: Bordeaux mixture (1%)+ Carbofuran- 3G (15 g) (349.33 nematodes/200 cc soil) and T11: *P. lilacinum* (50 g) (371.33 nematodes/200 cc sol) as compared to untreated control (922.00 nematodes/200 cc soil) respectively. The final root population of *R. similis* was minimum in T4: Bordeaux mixture (1 %) + *P. lilacinum* (50 g) (150.66/5 g roots) followed by T13: Carbofuran 3G (15 g) (178.00 /5 g roots nematodes) as compared to untreated control (478.00/5 g roots) respectively. The lowest foliar yellowing, defoliation and lesion indices were observed in the treatment T4: Bordeaux mixture (1 %) + *P. lilacinum* (50 g) (1.33, 1.00 and 1.00) followed by T2: Bordeaux mixture (1 %) + *T. harzianum* (50 g) (1.66, 1.33 and 1.00) and these two treatments were on par with each other. Vines treated with Bordeaux mixture (1 %) spray + *T. harzianum* (50 g) recorded maximum dry berry weight of 2.27 g / vine and it was on par with vines treated with Bordeaux mixture (1 %) + *P. lilacinum* (50 g) with 2.05 g/vine. However, untreated control vines recorded lowest dry berry weight (0.63 g) and it was on par with Carbofuran 3G (15 g) (1.04g).

Keywords: *Burrowing nematode, black pepper, Trichoderma harzianum, P. lilacinum*

Citation: N. Umashankar Kumar, *et al*., (2018) Ecofriendly Management of Wilt complex in Black pepper (*Piper nigrum* L.). International Journal of Agriculture Sciences, ISSN: 0975-3710 & E-ISSN: 0975-9107, Volume 10, Issue 20, pp.- 7415-7420.

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Introduction

Black pepper (*Piper nigrum* L.), (Family Piperaceae) known as the "King of Spices" has remained most precious and valuable spice in the world. It is also called as "Black gold" due its durability and value. It is playing a vital role in International trade. It is said that the European invaded India primarily for this very spice. Black pepper is native to India and is extensively cultivated in tropical regions. Currently, Vietnam is the world's largest producer and exporter of black pepper, producing 34 percent of the world's demand [1]. The cultivation of black pepper is mainly confined to India, Brazil, Indonesia, Malaysia, Thailand, Sri Lanka and Vietnam. During 2013-14, 21,250 tonnes of black pepper worth Rs. 94,002 lakhs were exported to various countries [2]. Black pepper is cultivated to a large extent in Kerala, Karnataka and Tamilnadu and to a limited extent in Maharashtra, north eastern states and Andaman & Nicobar Islands and Pondicherry. Kerala and Karnataka account for a major portion of production of black pepper in the country and to a lesser extent, in Maharashtra, Andhra Pradesh, Tamil Nadu and north eastern regions [1]. Slow wilt of black pepper is a debilitating disease where the affected plants survive for several years and death of plant occurs gradually over a period of 3-4 years. This disease has been referred to under different names such as slow wilt or slow decline in India, yellow or yellows disease in Indonesia. The drastic drop in the black pepper production in India has been attributed mainly for pronounced mortality of vines by the dreaded foot rot caused by *Phytophthora capsici* and nematodes, *Meloidogyne incognita*, *Radopholus similis*. The other major constraints for low production of black pepper are old gardens occupied with traditional cultivars having poor genetic potential, non-adoption of improved package of practices and bad management of gardens. *R. similis* and *M. incognita* are the primary incitants of slow decline in black pepper, though *P. capsici* can also induces similar symptoms [3]. Slow decline (Slow wilt or yellows disease) causes up to 32 percent crop loss in Indonesia [4] and about 30 percent vines are damaged annually in Guyana by this disease [5].

Unfortunately, in spices still nematicides have been the primary option for growers in managing nematodes. There is an urgent need for the development of a non chemical and eco friendly control options. Such work is necessary to develop eco friendly management practices and minimize chemical use while maintaining high production standards. By keeping these factors, the present study was taken to manage effectively by using biocontrol agents.

Review of literature

Integrated disease management would be the ideal strategy to tackle the complex and elusive soil borne problems like foot rot of black pepper, since single approach would be of little consequence to contain the disease. Nursery hygiene, phytosanitation and other cultural practices, chemical control, biocontrol measures coupled with host resistance are important components of integrated disease management that would reduce the pesticide load into the environment. Out of the various components of integrated disease management, biocontrol programmes are of high priority in managing soil borne plant pathogens. An integrated approach with cheap and efficient plant protection technology is of great relevance to check plant parasitic nematodes and *P. capsici*. A survey and revealed that the heavy incidence of root knot nematode in all black pepper growing areas of Karnataka and recorded gall indices up to 4 and further reported that where root knot nematode was observed, the *Phytophthora* infection was also more leading to devastating disease complex called slow wilt diseases [6]. There are indeed several reports of the joint occurrence of *Meloidogyne incognita* and *Fusarium* spp. in the roots of black pepper plants in Brazil. Infected plants showed wilting, yellowing of leaves, rotting of roots and stems and cracking of stems [7]. A field trial on management of slow decline of black pepper (*Piper nigrum* L.) in arecanut + black pepper mixed cropping system was taken up with sail application of Phorate, Neem cake and Carbendazim. Application of nematicides and neem cake was highly effective in reducing the populations of *Meloidogyne incognita* and *Radopholus similis* [3]. *Paecilomyces lilacinum* principally infects and assimilates eggs of root knot nematode and cyst nematodes. The fungus has been considered to have the greatest potential for application as a biocontrol agent in subtropical and tropical agricultural soils [8]. The efficacy of *Paecilomyces lilacinum* in suppressing root-knot nematode (*Meloidogyne incognita*) and burrowing nematode (*Radopholus similis*) infestations in black pepper (*Piper nigrum*) was studied. Though the fungus could not affect absolute control of nematodes, it significantly suppressed nematode infestation and increased total root mass production. The fungus was more effective in suppressing *M. incognita* than *R. similis* [9]. The role of biocontrol and biofertilizers for production of black pepper which has great demand in global market. Hence, identification of potential bioagents and bio fertilizers in disease control as well as improving the soil health assumes top priority in the context of organic production [10]. Use of a biocontrol agent (*Trichoderma harzianum*) for *Phytophthora* foot rot in black pepper. Parameters such as yield increase (*i.e.* quantity saved), change in cost of cultivation and improvements in economic returns were used to assess the impact of the project. Adoption of the technology resulted in maximum proportionate productivity increase of 11.6 percent and the net proportionate reduction in cost per ton output was 78.3 percent [11]. Maximum Shoot length, shoot weight and root length were significantly increased in mixture treatments compared to individual treatments, principally combination of seedling treatment (10 g/L water) + soil application treatment (5.0 kg/acre) documented maximum shoot length (60 and 90 DAS), shoot weight (90 DAS) and root length (90DAS) and they were positively correlated with fruit yield of okra [12]. Two kg of *P. putida* or *P. lilacinus* were used for the enrichment of vermicompost (200 kg) which was applied to the beds at the rate of 50 g/m^2 as a substrate treatment before sowing the corms. Corms of Gladiolus (cv. White prosperity) were also treated with the suspension of *P. putida* and *P. lilacinus* enriched in vermicompost for 5-10 min. Results indicated that combination treatment of *P. putida* (2x10-8/ml) and *P. lilacinus* (2x10-6 /ml) was more effective when compared with all other treatments [13]. Integrated disease management of wilt complex in black pepper and reported that foliar spraying of 1% Bordeaux mixture during and May and June followed by drenching and spraying with same fungicide during October coupled with soil application of *Trichoderma harzianum* around the base of the vine @ 50 g/vine during May - June and October months [2].

Material and methods

The present Experiment was conducted in the garden of farmer Sri. Puttegowda, G. Hosally village, Gonibeedu hobli, Mudigere taluk, Chickmagalur district. The field was heavily infested with *R. similis* at population density of more than one nematode per g soil *i.e.*, 864 nematodes per 200cc soil. Farm was situated in Agro climatic zone-9 (Hilly region Zone) of Karnataka state at varied elevations (900-950msl) and rainfall (2500 to 3000mm) with 110 56' and 150 46' N latitude and 740 31' and 760 46' E longitude. Further the experiment was conducted in the existing 10-12 years of old orchard, which was grown as mixed crop along with arecanut and forest plants. Farm Yard Manure @ 10 kg /vine was commonly applied for all the treatments and normal package of practices like irrigation, fertilizer application and weeding were done uniformly to all the plants by the farmer of the field.

Experimental details

Crop : Black pepper
Cultivar : Panniyur – 1
Design : RCBD
Treatments : 14
Replications : 3

Treatment Details

T1: Bordeaux mixture spray (1%)
T2: T1 + *Trichoderma harzianum* (50g)
T3: T1 + *Pseudomonas fluorescens* (50g)
T4: T1 + *Purpureomyces lilacinum* (50g)
T5: T1 + *Bacillus subtilis* (50g)
T6: T1 + Carbofuran 3G–15gms
T7: T1 + Neem cake 2 kgs
T8: Neem cake 2 kgs
T9: *Pseudomonas fluorescens* (50g)
T10: *Trichoderma harzianum* (50g)
T11: *Purpureomyces lilacinum* (50g)
T12: *Bacillus subtilis* (50g)
T13: Carbofuran 3G–15g
T14: Untreated Control

The talc-based formulations of *T. harzianum*, *P. lilacinum P. fluorescens*, and *B. subtilis* were obtained from Indian Institute of Horticultural Research (IIHR), Bangalore. The experiment was conducted in two seasons' *viz*., 2014-15 and 2015-16 and biocontrol agents were applied before and after monsoon along with 10 kgs of farm yard manure. Organic amendments and Bordeaux mixture was sprayed before and after monsoon every year.

Observations recorded

1. **On host:** Number of runner shoots, Number of spikes, Spike length, Spike weight, dry weight (per vine)
2. **On nematode:**
I. Initial nematode population before the treatment imposition
II. Nematode population at 60 days intervals
III. Final nematode population
IV. Number of lesions per plant
V. Root lesion index
3. **On wilt complex incidence**:
I. Number of wilted plants
2. Percent defoliation index (1-4)
3. Percent leaf yellowing index (1-4)
4. Percent root necrosis (0-5)
5. Leaf lesion index (0-4)
6. Root lesion index (1-5)

The total number of plants showing foliar yellowing symptoms and defoliation were recorded by using the following scale (Mohandas and Ramana, 1991).

Foliar Yellowing Index (FYI): 1-4 scale

S	Scale	Descriptions
1	1	No leaves showing yellowing
2	2	Up to 20 per cent of leaves showing yellowing
3	3	20-60 per cent leaves showing yellowing
4	4	More than 60 per cent leaves showing yellowing

Defoliation Index (DFI): 1-4 scale

S	Scale	Descriptions
1	1	Less than 10 per cent defoliation
2	2	More than 10 per cent upto 30 per cent defoliation
3	3	More than 30 per cent upto 60 per cent defoliation
4	4	More than 60 per cent defoliation

The virulence rating of *P. capsici* in green house condition was done at 10 days interval till the termination of the experiment using the following disease rating scale by Turner (1973) based on percent root necrosis.

Grade	Root necrosis (%)
0	0
1	1-10
2	11-25
3	26-50
4	51-75
5	76-100

Lesion Index: The root lesion index was calculated using the lesion index rating scale.

Lesion number and size	Lesion index
No lesions	1
Few up to 1mm diameter (1-20 lesions)	2
Many up to 1mm diameter (21-50 lesions)	3
Many upto 1cm diameter (51-100 lesions)	4
Very severe 1 cm diameter (>100 lesions)	5

While collecting soil and root samples lesion characters were also recorded. The wilt disease incidence in the fields was calculated by using following formula.

$$Disease\ incidence\ (\%)\ = \frac{Number\ of\ plants\ infected}{Total\ Number\ of\ plants\ observed} \times 100$$

Soil sample of 200 cc was washed thoroughly and processed using combined Cobb Sieving and Baermann's funnel technique (Ayoub, 1977) as given below.

Procedure

1. Two hundred cc of soil was taken in 1000 ml beaker and sufficient quantity was added to make soil solution.
2. This was stirred thoroughly and allowed to stand for a minute for the heavier particles to settle down.
3. Then the soil solution was passed through a set of sieves of 100, 250, 325 and 400 mesh sizes, respectively.
4. Residues from 325 and 400 mesh sieves were collected and poured over tissue paper on a wire gauge placed on a Baermann's funnel.
5. Level of water in the funnel was maintained to keep the tissue paper wet and left undisturbed for 48 hours.
6. After incubation of 48 hr, the volume of suspension was made to 200 ml, out of which 10 ml was taken and used for counting of various plant parasitic nematodes present.
7. Suspension of water in the funnel was collected and nematodes were observed by using stereo binocular microscope.

Processing of roots and estimation of nematode population

Nematode population in 5 g of roots was estimated by root incubation method (Ayoub, 1977) as explained below:

Procedure

1. Roots were gently washed to remove adhering soil particles.
2. Washed roots were cut into small bits of 2.5cm and split longitudinally.
3. Then placed over tissue paper spread on a wire gauge and kept in a Petri plate filled with water.
4. Level of water was maintained in Petri plate and left undisturbed for 48 hours.
5. Later, the suspension in the Petri plate was collected and observed for nematodes using stereo binocular microscope.

Results and discussion

The field experiment was carried out in the farmer's field for two years, with a view to evaluate bioagents (*Trichoderma harzianum*, *Purpureomyces lilacinum*, *Pseudomonas fluorescens* and *Bacillus subtilis*), organic amendment (Neem cake) and chemicals (Carbofuran and Bordeaux mixture) separately and in combination for the management of *R. similis* and *P. capsici*.

Observations on plant growth parameters like Number of runner shoots, Number of spikes, Spike length, Spike weight, and dry weight (per vine) was calculated and presented in the [Table-1].

Number of runner shoots

Among combination treatments of bioagents, organic amendments and chemicals, maximum number of runner shoots was observed in plants with Bordeaux mixture (1 %) spray + soil application of *T. harzianum* (50 g) with 27.33 followed by Bordeaux mixture (1 %) + *P. lilacinum* (50 g) recorded 25.00 and it was on par with 24.33 number of runner shoots in Bordeaux mixture (1 %) + *P. fluorescens* (50 g). lowest number of runner shoots was observed in Bordeaux mixture (1 %) + Carbofuran-3G (15 g) treated vines with 19.66 number of runner shoots and it was on par with Bordeaux mixture (1 %) + Neem cake (2 kg) with 20.33 number of runner shoots.

Among the individual treatments, maximum number of runner shoots (16.33) was observed in *T. harzianum* (50 g) and lowest number of runner shoots (7.33) in untreated control.

Number of spikes

Highest number of spikes was produced in the vines treated with Bordeaux mixture (1 %) +*T. harzianum* (50 g) with 321.33 followed by Bordeaux mixture (1 %) + *P. lilacinum* (50 g) which recorded 294.66 number of spikes and lowest number of spikes was produced in untreated control plots (89.33) followed by 151.33 in Carbofuran-3G (15 g) respectively.

Spike length (cm)

With respect to spike length, all the treatments were on par with each other. Vines treated with Bordeaux mixture (1 %) +*T. harzianum* (50 g) and Bordeaux mixture (1 %) + *P. lilacinum* (50 g) recorded maximum spike length of 17.66 cm respectively and it was on par with vines treated with T3: Bordeaux mixture (1 %) + *P. fluorescens* (50g) (16.66 cm) and Bordeaux mixture (1 %) + *B. subtilis* (50 g) (15.66 cm). Lowest spike length was observed in vines with untreated control (7.66 cm) and it was on par with Carbofuran 3G (15 g) (8.33 cm) and *B. subtilis* (50 g) (8.66 cm).

Spike weight

Highest spike weight was recorded in Bordeaux mixture (1 %) + *T. harzianum* (50 g) with 2992.00 g followed by Bordeaux mixture (1 %) + *P. lilacinum* (50 g) with 2760.66 g and lowest spike weight was recorded in untreated control (1072.66 g) and it was on par with Carbofuran 3G (15 g) (1241.66 g).

Dry berry weight per vine (Kgs)

Vines treated with Bordeaux mixture (1 %) spray + *T. harzianum* (50 g) recorded maximum dry berry weight of 2.27 g / vine and it was on par with vines treated with Bordeaux mixture (1 %) + *P. lilacinum* (50 g) with 2.05 g/vine. However, untreated control vines recorded lowest dry berry weight (0.63 g) and it was on par with Carbofuran 3G (15 g) (1.04 g).

Effect of various treatments on nematode population in soil and roots of black pepper infected by *R. similis* and *P. capsici* at different intervals (Season-I)

Nematode population in soil and roots

The *R. similis* population in soil and roots was recorded at 60 days interval from treatment imposition to harvest. The population of *R. similis* in soil differed significantly among all the treatments compared to control. The data is presented in [Table-2]. Among the individual treatments, lowest multiplication of *R. similis* was observed in Carbofuran 3G treatment followed by *P. lilacinum* and *T. harzianum*, the same trend was followed in all the intervals of sampling till the harvest of the crop. Among combined treatments, the T2: Bordeaux mixture (1 %) + *T. harzianum* (50 g), T3: Bordeaux mixture (1 %) + *P. fluorescens* (50 g), T4: Bordeaux mixture (1 %) + *P. lilacinum* (50 g), T5: Bordeaux mixture (1 %) + *B. subtilis* (50 g), T6: Bordeaux mixture (1 %) + Carbofuran-3G (15 g) and T7: Bordeaux mixture (1 %) + Neem cake (2 kg) the soil was maximum in *Bacillus subtilis* treated vines followed by *P. fluorescens* at all the intervals. The population of *R. similis* in roots was differed significantly among the treatments compared to control. The same trend was observed in root population as that of soil population. The final population of *R. similis* in soil was lowest in T4: Bordeaux mixture (1 %) + *P. lilacinum* (50 g) (302.66/200 cc soil), followed by T6: Carbofuran 3G (15 g) (335.33 nematodes/200 cc soil), T13: Bordeaux mixture (1%)+ Carbofuran- 3G (15 g) (349.33 nematodes/200 cc soil) and T11: *P. lilacinum* (50 g) (371.33 nematodes/200 cc sol) as compared to untreated control (922.00 nematodes/200 cc soil) respectively. The final root population of *R. similis* was minimum in T4: Bordeaux mixture (1 %) + *P. lilacinum* (50 g) (150.66/5 g roots) followed by T13: Carbofuran 3G (15 g) (178.00 /5 g roots nematodes) as compared to untreated control (478.00/5 g roots) respectively.

Table-1 Influence of various treatments on growth and yield parameters of black pepper under field conditions (Season-I)

Treatments	Number of runner shoots	Number of spikes	Spike length (cm)	Spike weight (g)	Dry berry weight/ vine (Kg)	Yield Kg/ha
T_1: Bordeaux mixture (1 %) spray	11.66	183.33	10.33	1316.33	1.27	254.66
T_2: T_1 + *T. harzianum* (50 g)	27.33	321.33	17.66	2992.00	2.27	453.33
T_3: T_1+ *P. fluorescens* (50 g)	24.33	285.66	16.66	2516.66	1.91	381.33
T_4: T_1+ *P. lilacinum* (50 g)	25.00	294.66	17.66	2760.66	2.05	411.33
T_5: T_1+ *B. subtilis* (50 g)	22.33	284.66	15.66	2309.33	1.70	340.66
T_6: T_1+ Carbofuran-3G (15 g)	19.66	213.33	11.66	2016.00	1.59	317.33
T_7: T_1+ Neem cake (2 kg)	20.33	280.00	14.33	2189.33	1.83	366.66
T_8: Neem cake (2 kg)	11.00	179.33	9.33	1316.33	1.36	271.33
T_9: *P. fluorescens* (50 g)	13.33	191.33	9.00	1511.00	1.48	296.66
T_{10}: *T. harzianum* (50 g)	16.33	214.66	12.33	1968.00	1.59	319.33
T_{11}: *P. lilacinum* (50 g)	14.66	203.33	11.33	1778.66	1.41	282.66
T_{12}: *B. subtilis* (50 g)	12.33	163.33	8.66	1164.66	0.82	164.66
T_{13}: Carbofuran 3G (15 g)	10.66	151.33	8.33	1241.66	1.04	208.00
T_{14}: Untreated	7.33	89.33	7.66	1072.66	0.63	127.33
S. Em ±	0.52	24.18	0.92	135.72	0.27	2.93
CD @ 5 %	1.50	70.29	2.67	394.63	0.77	8.54
CV (%)	5.29	19.18	13.06	12.39	29.35	1.69

Table-2 Effect of various treatments on population of *R. similis* in soil and roots of black pepper at different intervals under field conditions

Treatments	Nematode population (200 cc soil) Days after treatment				Nematode population (5 g roots) Days after treatment			
	60	120	180	240	60	120	180	240
T_1 : Bordeaux mixture (1 %) spray	632.00	618.00	558.66	543.33	303.33	324.00	338.00	290.66
T_2 : T_1 + *T. harzianum* (50 g)	594.00	516.66	482.00	389.33	237.33	276.00	241.33	179.33
T_3: T_1+ *P. fluorescens* (50 g)	610.66	552.66	510.66	451.33	278.00	300.66	281.33	203.33
T_4 : T_1+ *P. lilacinum* (50 g)	555.33	505.33	418.00	302.66	222.66	267.33	249.33	150.66
T_5 : T_1+ *B. subtilis* (50 g)	626.00	596.00	501.33	417.33	280.00	317.33	304.00	230.66
T_6 : T_1+ Carbofuran-3G (15 g)	489.33	432.00	402.66	335.33	179.33	235.00	214.66	184.66
T_7 : T_1+ Neem cake (2 kg)	608.66	530.00	476.00	412.00	256.00	291.33	265.33	191.33
T_8 : Neem cake (2 kg)	618.00	561.33	434.00	430.00	260.00	297.33	273.33	196.00
T_9 : *P. fluorescens* (50 g)	600.00	570.66	503.33	444.66	276.33	308.66	287.33	210.00
T_{10} : *T. harzianum* (50 g)	583.33	531.33	494.66	401.33	252.00	282.00	259.33	190.00
T_{11} : *P. lilacinum* (50 g)	578.66	493.33	464.66	371.33	230.66	251.33	232.66	163.33
T_{12} : *B. subtilis* (50 g)	619.99	583.33	520.00	463.33	264.00	309.33	296.66	219.33
T_{13} : Carbofuran 3G (15 g)	519.33	477.33	384.66	349.33	215.33	244.00	226.66	178.00
T_{14} : Untreated	699.33	814.00	883.33	922.00	338.66	405.33	435.33	478.00
S. Em ±	3.89	31.45	3.92	2.75	17.91	1.80	1.49	2.96
CD @ 5 %	11.33	91.47	11.39	8.01	52.09	5.25	4.34	8.61
CV (%)	1.13	9.77	1.35	1.07	12.08	1.06	0.93	2.34

INP: 864 J_2/200 cc soil

Table-3 Effect of various treatments on foliar yellowing, defoliation and lesion indices on balck pepper under field conditions

Treatments	Foliar yellowing (1-4)	Defoliation index (1-4)	Leaf Lesion index (0 - 4)
T_1: Bordeaux mixture (1 %) spray	2.66	2.33	1.33
T_2: T_1 + *T. harzianum* (50 g)	1.66	1.33	0.66
T_3: T_1+ *P. fluorescens* (50 g)	3.00	3.00	1.66
T_4: T_1+ *P. lilacinum* (50 g)	1.33	1.00	1.00
T_5: T_1+ *B. subtilis* (50 g)	4.00	3.33	2.33
T_6: T_1+ Carbofuran-3G (15 g)	4.00	3.66	2.33
T_7: T_1+ Neem cake (2 kg)	2.66	2.33	2.00
T_8: Neem cake (2 kg)	3.33	2.66	2.33
T_9: *P. fluorescens* (50 g)	3.33	2.66	2.66
T_{10}: *T. harzianum* (50 g)	2.33	1.66	2.66
T_{11}: *P. lilacinum* (50 g)	2.00	1.66	2.00
T_{12}: *B. subtilis* (50 g)	4.00	3.00	3.00
T_{13}: Carbofuran 3G (15 g)	4.00	3.66	3.66
T_{14}: Untreated	4.00	4.00	4.00
S. Em ±	0.23	0.23	0.31
CD @ 5 %	0.69	0.67	0.91
CV (%)	13.60	15.96	24.00

Effect of various treatments on wilt complex incidence in black pepper infected with *R. similis* and *P. capsici*

Influence of bioagents, organic amendments and chemicals on foliar yellowing, defoliation and leaf lesion indices were recorded before harvest and the data is presented in [Table-3]. The lowest foliar yellowing, defoliation and lesion indices were observed in the treatment T4: Bordeaux mixture (1 %) + *P. lilacinum* (50 g) (1.33, 1.00 and 1.00) followed by T2: Bordeaux mixture (1 %) + *T. harzianum* (50 g) (1.66, 1.33 and 1.00) and these two treatments were on par with each other. Highest foliar yellowing, defoliation and lesion indices were observed in untreated control (4.00, 4.00 and 4.00) followed by T13: Carbofuran 3G (15 g) (4.00, 3.66 and 3.66) respectively.

Table-4 Effect of various treatments on *R. similis* and *P. capsici* under field conditions

Treatments	No. of lesions	Lesion index (1- 5)	Root necrosis (%)	Wilt incidence (%)	% decrease over control	Yield/ Vine (Kg)	Percent increase over control	B: C Ratio
T_1: Bordeaux mixture (1 %) spray	116.00	5.00	32.00	28.33	64.28	1.27	101.58	1.19
T_2: T_1 + *T. harzianum* (50 g)	60.00	4.00	26.67	13.33	83.19	2.27	260.03	2.92
T_3: T_1+ *P. fluorescens* (50 g)	73.66	4.00	39.33	38.00	52.09	1.91	203.17	1.61
T_4: T_1+ *P. lilacinum* (50 g)	51.33	3.66	23.00	13.66	82.78	2.05	225.39	2.48
T_5: T_1+ *B. subtilis* (50 g)	97.00	4.00	64.66	48.66	38.66	1.70	169.84	1.33
T_6: T_1+ Carbofuran-3G (15 g)	65.00	4.00	35.67	23.00	71.00	1.59	152.38	1.24
T_7: T_1+ Neem cake (2 kg)	89.67	4.00	48.66	33.66	57.56	1.83	190.47	1.60
T_8: Neem cake (2 kg)	106.00	5.00	74.00	55.66	29.83	1.36	115.87	1.46
T_9: *P. fluorescens* (50 g)	106.66	5.00	69.33	61.33	22.69	1.48	134.92	1.30
T_{10}: *T. harzianum* (50 g)	96.66	4.00	53.00	42.00	47.06	1.59	152.38	1.38
T_{11}: *P. lilacinum* (50 g)	80.67	4.00	44.66	42.33	46.66	1.41	123.80	1.24
T_{12}: *B. subtilis* (50 g)	110.33	5.00	75.33	69.33	12.60	0.82	30.15	0.73
T_{13}: Carbofuran 3G (15 g)	82.66	4.00	59.00	74.66	5.89	1.04	65.07	0.91
T_{14}: Untreated	146.67	5.00	83.66	79.33	0.00	0.63	0.00	0.71
S. Em ±	1.16	0.09	0.68	0.82	-	0.27	-	0.02
CD @ 5 %	3.37	0.26	1.98	2.38	-	0.77	-	0.05
CV (%)	2.19	3.50	2.26	3.18	-	29.35	-	2.12

Effect of various treatments on multiplication of nematodes and percent root necrosis on black pepper infested with *R. similis* and *P. capsici*

Influence of bioagents, organic amendments and chemicals on number of lesions, lesion index and percent root necrosis on black pepper was analyzed before harvest and the data is presented in [Table-4].

Number of lesions and lesion index

There was a significant differences observed between the treatments in number of lesions on roots and the lowest number of lesions and lesion index was observed in the T4: Bordeaux mixture (1 %) + *P. lilacinum* (51.33 and 3.66), T2: Bordeaux mixture (1 %) +*T. harzianum* (60.00 and 4.00) and T6: Bordeaux mixture (1 %) + Carbofuran (65.00 and 4.00) respectively. Highest number of lesions and lesion index were observed in untreated control (146.00 and 5.00) followed by Bordeaux mixture (1 %) (116.00 & 5.00).

Percent root necrosis

Similar trend was also followed in percent root necrosis and lowest root necrosis was recorded in T4: Bordeaux mixture (1%) + *P. lilacinum* (23.00 %), followed by T2: Bordeaux mixture (1 %) +*T. harzianum* (26.67 %) and these two treatments were on par with each other. Highest percent root necrosis was observed in untreated control (83.66 %) followed by T12: *B. subtilis* (75.33 %).

Percent wilt incidence

The results indicated that, plants treated with T2: (Bordeaux mixture (1 %) + *T. harzianum* (50 g) recorded very less wilt incidence of 13.33 % compared to the maximum wilt incidence (79.33 %) in untreated control. The next best treatment was plant inoculated with T4: (Bordeaux mixture (1 %) + *P. lilacinum* (50 g), which recorded the wilt incidence (13.66 %) which was on par with T2: Bordeaux mixture (1 %) +*T. harzianum* reduced the wilt incidence compared to individual application of bioagents [Table-4].

Influence of various treatments on yield and economic benefit of disease management in black pepper infested with *R. similis* and *P. capsici*

All the treatments recorded increased yield and B: C ratio with and decreased wilt incidence and the data is presented in [Table-4]. Individual application of *P. lilacinum* (1.41 kg), *T. harzianum* (1.59 kg), *P. fluorescens* (1.48 kg), *B. subtilis* (0.82 kg), neem cake (1.36 kg), Bordeaux mixture (1 %) spray (1.27 kg) and Carbofuran (1.04 kg) and these treatments did not significantly differ in yield per vine and the lowest yield was recorded in untreated control (0.63 kg/vine). Among the combined treatments, maximum yield (2.27 kg) was recorded in plants treated with Bordeaux mixture (1 %) +*T. harzianum* (50 g) followed by 2.05 kg in Bordeaux mixture (1 %) + *P. lilacinum* (50 g) treated plants. The economic analysis, for integrated management of *R. similis* and *P. capsici* wilt on black pepper under field conditions was carried out. The results revealed that, the T2: Bordeaux mixture (1 %) +*T. harzianum* (50 g) recorded maximum B: C ratio (2.92) with wilt lowest incidence of 13.33 percent followed by T4: Bordeaux mixture (1 %) + *P. lilacinum* (50 g) recorded B: C ratio (2.48) and percent disease incidence (13.66 %) and these two treatments were on par with each other and superior over other treatment. It was apparent that, the integration application of Bordeaux mixture (1 %) +*T. harzianum* (50 g) or Bordeaux mixture (1 %)+ *P. lilacinum* was most effective in improving plant growth parameters, berry yield and in reducing nematode population, percent yellowing, defoliation, leaf lesion indices and percent root necrosis and also maximum B: C ratio. Integrated disease management would be the ideal strategy to tackle the complex and elusive soil borne problems like foot rot of black pepper, since single approach would be of little consequence to contain the disease. Nursery hygiene, phytosanitation and other cultural practices, chemical control, biocontrol measures coupled with host resistance are important components of integrated disease management that would reduce the pesticide load into the environment. Out of the various components of integrated disease management, biocontrol programmes are of high priority in managing soil borne plant pathogens. An integrated approach with cheap and efficient plant protection technology is of great relevance to check plant parasitic nematodes and *P. capsici*. The results revealed that combination of Bordeaux mixture (1%) and *T. harzianum* (50 g) application or combined applications of Bordeaux mixture (1%) + *P. lilacinum* (50 g) provide the maximum growth and yield of black pepper infested with *R. similis* and *P. capsici* under filed conditions. The present findings are in confirmation with findings of [19] they, reported that the addiction of *Paecilomyces lilacinus* and *Trichoderma harzianum* as nematophagous fungi separately along with organic manure to the infested field sufficiently retarded the pathogenic activity of *M. incognita* and increases plant vigor. The present investigations were similar to that of number of roots and biomass production were higher with combined application of *P. fluorescens* (thrice) and *T. harzianum* which was on par with application of *P. fluorescens* thrice [10]. Use of a biocontrol agent (*Trichoderma harzianum*) for *Phytophthora* foot rot in black pepper, parameters such as yield increase (*i.e.* quantity saved), change in cost of cultivation and improvements in economic returns were used to assess the impact of the project. Adoption of the technology resulted in maximum proportionate productivity increase of 11.6 % and the net proportionate reduction in cost per ton output was 78.3 % [11]. Combined treatment of *P. putida* (2X10-6/ml) and *P. lilacinus* (2X10-6/ml) was more effective when compared with all other treatments. These treatments increased the plant growth and rhizospheric colonization of both bioagents significantly and reduced the disease incidence of *M. incognita* and *F. oxysporium* f. sp. gladioli by 66 percent and 57 percent respectively. There was also a significant increase in the yield of the crop which was to the tune of 23 percent (13). Dipel (*B. thuringiensis*) & Bio-nematon (*Paecilomyces lilacinus*) showed their superiority on the shoot, root length and root weight (14). This performance of fungal bioagents in the present study may be attributed to their strong fungicidal and nematicidal property against both the pathogens which provided maximum defense with improved plant growth. The various phytoharmones produced by PGPR play a major role in growth promotion and many bacteria have the ability to produce auxins, gibberlines and cytokinines and ethylenes [15]. Similarly, *T. harzianum* and is potential biocontrol agent which poses growth hormone and all these combinations resulted in improved plant growth parameters. The *T. viridae* has been reported to be a natural source of enzymes and plant hormones provide additional support to plants for its better growth development and immunity [20].

Nematode population and wilt incidence

With respect to nematode population in soil plants treated with Bordeaux mixture (1 %) + *P. lilacinum* (50 g) recorded lowest nematode population compared to other treatments and it was significantly superior over other treatments. The efficacy of *Paecilomyces lilacinum* in suppressing *M. incognita, R. similis* infestations in black pepper (*Piper nigrum*) was studied. Though the fungus could not affect absolute control of nematodes, it significantly suppressed nematode infestation and increased total root mass production [3]. Biocontrol agent *Trichoderma harzianum* along with potassium phosphonate has recorded highest disease suppression with least foliar yellowing [16]. The results demonstrated that, *P. lilacinum* is an effective biocontrol agent against *R. similis* in banana and can be an important component of integrated pest management strategies [17]. The present studies also in similar findings with [18] and reported that less foliar yellowing (13.52 %), less defoliation (15.28 %), less death of vines (4.72 %) and highest green berry yield of 2.46 Kg per vine when vines were treated before onset of monsoon (May), during rainy season (June- July) and during 2nd fortnight of August with potassium phosphonate (0.3 %) as spraying (2 l/vine) and drenching of *Trichoderma harzianum* 50g pel vine with 1kg of neem cake to the root zone. This is followed by chemical check with application of (1.0 %) Bordeaux mixture spray (2 L /vine) and copper oxychloride (0.1 %) as drenching (3l/vine) where in less foliar yellowing (16.6%), less defoliation (20.25 %), less death of vines (5.40 %) and green berry yield of 2.08 kg per vine were recorded.

Similar findings also observed that the antagonistic organism's *viz*., *Trichoderma viride*, *T. harzianum*, *Laetiseria arvalis*, and *Bacillus subtilis* were tested against *P. capsici* in pot culture by adding infected material to healthy vine. Among the four bioagents tried, *Trichoderam viride* and *T. harzianum* is effective in reducing the incidence of the disease as compared to *Laetiseria arvalis*, and *Bacillus subtilis*. The disease incidence was maximum in untreated vines [18]. Integrated disease management of wilt complex in black pepper and reported that foliar spraying of 1 % Bordeaux mixture during and May and June followed by drenching and spraying with same fungicide during October coupled with soil application of *Trichoderma harzianum* around the base of the vine @ 50 g/vine during May - June and October months [2]. The reasons for the reduced wilt incidence and increased yield may attributed to the Trichoderma spp. involved in the reduction of *P. capsici* by mycoparasitism, spatial and nutrient competition, antibiosis by enzymes and secondary metabolites and induction of plant defense system. The developing *P. lilacinum* kills the nematode by feeding on its body content and in effect *P. lilacinum* acts as a parasite on all the stages of nematode. The combined effects of these two bioagents against *R. similis* and *P.capsici* helped tremendously for the management of wilt complex in black pepper under field conditions. The amount of disease suppression obtained with a biological control agent depends on the density of the agent, the density of the pathogen and how efficiently individual units of the agents render units of the pathogen ineffective. Finally, it may be concluded that, soil borne pathogens like *P. capsici* and *R. similis* cannot be controlled with just a single management strategy. In the present study an integrated approach was attempted to manage this disease, with mixtures of biocontrol formulations which showed significant reduction in the disease incidence. The application of *P. lilacinum* and *T. harzianum* in combination with neem cake and farm yard manure is highly useful in managing *R. similis* and *P. capsici* wilt complex.

Application of research: The present investigation is useful for the farmers in controlling the wilt complex in organic farming and the biocontrol agents gives the effective control of the diseases for long lasting.

Research Category: Complex diseases by nematodes and fungus

Acknowledgement / Funding: Author thankful to University of Agricultural Sciences, GKVK, Bangalore, 560 065, India

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University: University of Agricultural Sciences, GKVK, Bangalore, 560 065, India
Research project name or number: PhD Thesis

Author Contributions: All authors equally contributed

Author statement: All authors read, reviewed, agreed and approved the final manuscript

Conflict of Interest: None declared

Ethical approval: This article does not contain any studies with human participants or animals performed by any of the authors.

References

[1] Anonymous (2005) www.icpnet.org.
[2] Devasahayam S., John Zacharaiah T., Jayashree E., Kandiannan E., Prasanth D., Santhosh J. E., Sasikumar B., Srinivasan V. and Suseela bhai R. (2015) *Black pepper, Extension Pamphlet, Indian institute of spice research, Kozhikode*. pp. 27.
[3] Ramana K. V., Sarma Y. R. and Mohandas C. (1992) *J. Plant. Crops*, 20, 65–68.
[4] Sitepu D. and Kasim R. (1991) *National Research Centre Spices, Calicut.*
[5] Biessar S. (1969) *PANS*, 15, 74-75.
[6] Raja Kumar N., Ravindra Kumar K. and Seshakiran K. (2012) *Green Farming*, 3 (5), 583-585.
[7] Koshy P. K., Santhosh J., Eapen S. J. and Pandey R. (2005) *CAB International, Wallingford, UK*, Pp. 751-792.
[8] Esser R.P. and Gholl N.E. (1993) *Paecilomyces lilacinus, a fungus that parasitizes nematode eggs. Nematology circular No. 203, Fla. Dept. Agric. And Consumer Services, Division of Plant industry, Gainesville.*
[9] Ramana K. V. (1994) *J. Spi. Arom. Cr*., 3, 130-134.
[10] Thankamani C. K., Sreekala K. and Anandaraj M. (2005) *J Spices and Aromatic Crops*, 14 (2), 112-116.
[11] Madan M. S., Mruthyunjaya Ramana K. V., Manoj K. A. and
[12] Anandaraj M. (2005) *Focus on Pepper*, 2 (1),1-20.
[13] Raja Kannan and Renganathan Veeravel (2012) *J. Agric. Sci*., 11,119-128.
[14] Sowmya D.S. and Rao M.S. (2013) *Hort. Eco*., 18 (2),204-209.
[15] Thankamani C. K., Srinivasan V. and Kandiannan K. (2004) *Spice India*, 47-49pp.
[16] Mohamed S. Khalil (2013) *T. E. Sci J. Plant Pathol*., 2, 84-91.
[17] Kumar A., Anandaraj M., Srinivasan V., Veena S. S. and Sarma Y.R. (2000) *Centennial Conference on Spices and Aromatic Plants, September,* 20-23,267-273.
[18] Mendoza A.R., Sikora R.A. and Kiewnick S. (2007) *Nematropica*, 37,203-213.
[19] Lokesh M.S. Patil S.V., Gurumurthy S.B., Palakshappa M.G. and Anandaraj M. (2013) *Int. J. Pl. Prot.* 6 (1), 139-141.
[20] Hafeez U.K. Riaz A., Waqar A., Khan S. M. and Aslam Khan M. (2001) *J. Biol. Sci*., 1 (3), 139-142.