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# EFFECT OF PHYTO-EXTRACTS AND BIO-AGENTS ON BIOCHEMICALS, ROOT ROT INCIDENCE AND *MELOIDOGYNE* POPULATION OF FENNEL (*FOENICULUM VULGARE* MILL.)

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**ABSTRACT**

Seed spices are very important for human health and widely grown throughout the country. Among these, fennel (*Foeniculum vulgare* Mill.) is one of the also important seed spices belong to the *Apiaceae* family. Root rot is the most common disease of fennel caused by *Fusarium solani* and its severity is aggravated by the presence of root rot nematode. In lieu of this, an experiment was conducted to manage *F. solani* in association with *Meloidogyne javanica* through bio-agents and phyto-extracts. The bio-agents (*Pseudomonas fluorescens* and *Trichoderma harzianum*) were applied through soil (2.5 kg/ha), while botanicals (garlic clove and parthenium leaves extract) through seed soaking (@10% for 30 min.). Maximum elevated growth parameters of fennel were recorded with *T. harzianum* + garlic extract followed by parthenium extract. The lowest root rot incidence (11.48%), reduced nematode population (163.47 eggs) and enhanced the level of PO (0.788 µmol/min/g), PPO (0.094 µmol/min/g), PAL (0.646 µmol/min/g) and phenol (3.110 mg/g) contents were recorded in *T. harzianum* + garlic extract treated roots of fennel plants over untreated check (0.423, 0.027, 0.117 µmol/min/g and 1.513 mg/g, respectively) followed by *T. harzianum* + parthenium extract. In conclusion, the use of the *T. harzianum* + garlic extract can be considered as a tool complementary for the commercial management of root rot of fennel in areas where this disease is gaining importance and becoming a limiting factor. Additionally, it can be used for managing pathogen resistance as well as also provide opportunity for organic production of spices for consumers.

***Key words :*** Fennel, Root rot, *Fusarium solani*, *Meloidogyne javanica*, Bio-agents, Phyto-extracts.

## Introduction

Among the seed spices, fennel (*Foeniculum vulgare* Mill.) is one of the important crops belongs to the *Apiaceae* family and originated from the Mediterranean region (Omidbaigi, 2000). In India, area under fennel cultivation is around 83 thousand hectares with a production of 137 thousand metric tonnes and productivity of 1.7 metric tonnes/ha, while in Rajasthan, area is around 30,814 hectares with 33,563 tonnes production and 1089 kg/ha productivity (Anonymous, 2020-21). During all the growth stages of fennel crop, right from germination to maturity is attacked by a large spectrum of diseases caused by fungi, bacteria, viruses and nematodes (Khare *et al*., 2014). Among diseases, root rot caused by *Fusarium solani* is a major fungal disease. The incidence of root rot was reported 20 to 25 per cent in fennel (Gupta *et al*., 1976). Root knot nematode, *Meloidogyne* spp. was reported on fennel and cumin crop (Patel, 2011; Kant

*et al*., 2013). Under tropical and subtropical conditions root knot nematode causes much higher damage (Taylor *et al*., 1978). In India, 21.07 per cent average loss caused by root knot nematode on fennel (Patel *et al*., 2002). There are several reports of complex disease development with nematode and fungus interaction in various crops *i.e*., tomato (Bhagawati *et al*., 2000; Meena *et al*., 2021a), pea (Akhtar *et al*., 2007), black gram (Kumar *et al*., 2017), fennel (Kumawat *et al*., 2022) and cucumber (Meena *et al*., 2023). During early phase of research, Atkinson (1892) stated that disease incidence of root rot may be increased by simultaneous or sequential infection by root knot nematodes and *Fusarium* spp. Later on, it was noted that nematodes facilitate fungus to invade plant roots and provoked disease severity by plant roots modification (Mai *et al*., 1987). Avoiding crop losses due to diseases caused by pathogenic fungi, bacteria, viruses, viroids and nematodes is one of the most significant issues in getting economic production of plant. At present, fast and effective control of fungal and other diseases in the crops is usually achieved using synthetic pesticides. Thus, chemical control of fungal diseases is largely dependent on the use of systemic and non-systemic fungicides. Furthermore, chemicals of this type evoke undesirable effects on the environment and leave residues, toxic to mammalians in the products and risk of developing resistance in microorganisms.

Many organic amendments, plant extracts and bio-agents or their combination are the most effective against the nematode fungus complex diseases in different crops. Soil population pathogen and root galling were significantly suppressed by application of bio-agents and botanicals. Therefore, some of the phyto-extracts and bioagents were tried to manage the disease considering environmentally safe and eco-friendly.

## Materials and Methods

### Botanicals

The leaves of Parthenium and garlic cloves were collected, washed water and grind in absolute alcohol in equal volume. The grinded material was kept at room temperature for 48 h and filtered through filter paper. The solvent, absolute alcohol was evaporated at 70ºC from the extract so as to get a semi-solid material. This semi-solid material was considered as stock solution for further experimentation with required concentration.

### Culture of *Fusarium solani*

The root rot causing fungus (*Fusarium solani*) wasa isolated from diseased roots of fennel and further purified and identified on the basis of cultural and morphological characters. For mass multiplication of fungus, sorghum grains were boiled partially and then spread on clean blotting paper for drying. About 250g of such sorghum grains were filled in each 1000ml flask and autoclaved at 15 psi pressure for 30 minutes. The mycelial bit (5 mm dia.) of 7 days old culture of *Fusarium solani* was inoculated under aseptic conditions on grains and incubated at 25±1$^0$C for 7 days. Meanwhile the flasks were jiggled to avoid clumping of grains and to facilitates early growth of fungus.

### Culture of *Meloidogyne javanica*

The fennel plants infected with root-knot nematode were collected from the field and brought to the laboratory. The infected roots were drenched in water and egg masses were carefully detached from roots. Later, infected roots having female nematodes were stained in 0.1 per cent acid fuchsin lactophenol and destained in pure lactophenol (McBeth *et al*., 1941). Perineal patterns of adult females were cut using a scalpel and removed the body contents using camel hair brush No. 1 (Taylor *et al*., 1974) and prepared a mount on glass slides. Observations of the perineal patterns showed the presence of *M. javanica* species. Simultaneously, collected egg masses were put into watch glasses in distilled water at laboratory temperature for hatching. Newly hatched 2$^{nd}$ stage juveniles ($J_2$) were then inoculated on already grown and maintained one month old fennel plants in pots filled with sterilized soil to acquired *M. javanica* pure population to carry out further experiments.

### Biochemical analysis

The determination of peroxidase (EC 1.11.1.7) activity (Hammerschmidt *et al*., 1982), polyphenol oxidase (EC 1.10.3.1) (Mayer *et al*., 1965), phenylalanine ammonia lyase (PAL) (EC 4.3.1.5) (Dickerson *et al*., 1984) and phenol activity (Malik and Singh, 1980) were estimated by following standard protocols.

### Bio-agents and botanicals in managing root rot disease and root-knot nematode

In this experiment, two bio-agents (*Pseudomonas fluorescens* and *Trichoderma harzianum*) and two phyto-extracts (garlic clove and parthenium leaves) were used alone as well as in combination. The bioagents were applied through soil (2.5 kg/ha) while botanicals through seed soaking (@10 % for 30 min.). Plants were uprooted sixty days after sowing and observations on growth characters, per cent root rot incidence, nematode reproduction and on accumulation of peroxidase, polyphenol oxidase, phenylalanine ammonia lyase and phenol were estimated in fennel roots using spectrophotometer.

**Statistical analysis**

Data were statistically analyzed for interpretation of findings. The critical deference was calculated for comparison of treatments at 5% level of significance.

## Results and Discussion

The data of the investigation indicated that all bio-agents (2.5 kg/ha in soil) and botanicals (seed soaking in 10 % for 30 min), applied alone or in combination, significantly increased plant growth parameters and decreased root rot incidence and nematode reproduction as compared to check (Table 1). Among these, soil application of *T. harzianum* (2.5 kg/ha) + seed soaking in garlic extract (10 % for 30 min) was recorded highly effective in providing maximum shoot length (25.50 cm), shoot weight (3.51 g), root length (21.94 cm), root weight (2.35 g) along with minimum root rot incidence (11.48 %), number of nematode galls/plant (6.67), number of egg masses/plant (3.67), number of eggs/egg mass (238.67) and number of juveniles/200 cc soil as compared to check (6.24cm, 0.43g, 4.70cm, 0.37g, 71.11%, 29.34, 23.34, 298.67 and 874.34, respectively). The present findings are in accordance with the results of earlier researchers (Kamal *et al*., 2009; Park *et al*., 2014; Meena *et al*., 2015; Arya, 2016; Baheti *et al*., 2016; Kumhar *et al*., 2018a & b; Bhati *et al*., 2022a & b). Kamal *et al*. (2009) observed effectiveness of neem oil in inhibiting root-knot nematode population in tomato while Park *et al*. (2014) suggested that *M. hapla* of tomato and carrot could be managed by application of biocontrol agent (*Bacillus cereus*). Similarly, reduced the infection of *M. incognita* and germination percentage in tomato increased with the combined application of *Trichoderma harzianum* and vermicompost (Arya, 2016). Use of plant extracts not only increase the maize growth but also decrease the nematode infection. Meena *et al*. (2015) applied neem leaves powders (4 q/ha) and found to be the most effective in improving growth of maize and reducing infection of *Heterodera zeae*. Baheti *et al*. (2016) observed neem leaves powder (@ 20 per cent w/w) as most effective in increasing maize growth and in reducing infection of cyst nematode in maize. Meena *et al*. (2016) have also reported that application of carbosulfan (@ 1% w/w) coupled with neem leaves powders (@ 1q/ha) was most effective in managing maize cyst nematode. Kumhar *et al*. (2018a & b) reported that use of *Calotropis procera* and *Pochonia chlamydosporia* (4%) increases the maize growth and reduces maize cyst nematode (*Heterodera zeae*). Improvement in efficacy of disease-control and crop yield was more economically and environmentally acceptable with integration of chicken manure with NPK, *T. harzianum* or *B. thuringiensis* (Osman *et al*., 2018). Meena *et al*. (2020) found that *Trichoderma viride* was superior in declining nematode population and per cent disease incidence along with increased growth of tomato. Similar results were also recorded with application of NSKE in tomato (Meena *et al*., 2021b). Hot water + tobacco churi + *Paecilomyces lilacinus* combination were significantly reduced nematode reproduction and enhanced cucumber growth (Bhati *et al*., 2021). Bhati *et al*. (2022a & b) also recorded increased cucumber yield with organic amendments and *Trichoderma viride* (5.0g per plant) along with reduced nematode reproduction.

The accumulation of peroxidase (PO), polyphenol oxidase (PPO), phenylalanine ammonia lyase (PAL) and phenol were estimated in fennel roots through spectrophotometer (Table 2). Results revealed that all the tested bio-agents and botanicals significantly enhanced the level of PO (0.788 µmol/min/g), PPO (0.094 µmol/min/g), PAL (0.646 µmol/min/g) and phenol (3.110 mg/g) activities in fennel roots over untreated control (0.423, 0.027, 0.117 µmol/min/g and 1.513 mg/g, respectively). Maximum enzymatic activity was found in *T. harzianum* + garlic treated plants followed by *T. harzianum* + parthenium and parthenium in comparison to untreated check. The present findings are in accordance with results of Singha *et al*. (2011), Zareena *et al*. (2014), Chandrawat *et al*. (2018, 2020 a & b) and Dhayal *et al*. (2022). Singha *et al*. (2011) observed higher phenolic accumulation in *Fusarium* infected plants in the comparison of healthy. Similarly, the application of *Pseudomonas fluorescens* and *Trichoderma viride* (@ 4g/kg soil) was the best in improving plant growth, reduction in root knot nematode (*M. incognita*) reproduction and increased PO, PPO, PAL and SOD activity of tomato (Chandrawat *et al*., 2018) as well as in chilli (Chandrawat *et al*., 2020a). Results were also found similar with application of castor, mahua, mustard and karanj oil-cakes (@ 2.5 and 5.0 q/ha) and neem (@ 5.0 q/ha) in tomato (Chandrawat *et al*., 2020b) in chilli (Chandrawat *et al*., 2022) against root knot nematode, *M. incognita*. Goel and Paul (2014) evaluated that disease severity were reduced in the neem extract treated plants by inducing activities of peroxidase and lipoxygenase. Report of various workers showed enhanced plant growth and reduced nematode population as well as galling of root with oil-cakes and bio-agents combined application. Effective results were also reported with integration of neem cake and VAM in brinjal (Bora *et al*., 2004). The integration of mustard, neem and castor oil-cakes with AMF (Bhardwaj *et al*., 2006);

**Table 1 :** Management of root rot disease of fennel and nematode population through bio-agents and botanicals.

| Bio-agents/ botanicals | Plant growth parameters | | | | Root rot incidence (%) * | Nematode reproduction** | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Shoot length (cm) | Shoot weight (g) | Root length (cm) | Root weight (g) | | No. of galls/plant | No. of egg masses/Plant | No. of eggs/ egg mass | Nematode juvenile/200cc soil |
| *T. harzianum* | 13.23 | 1.29 | 12.86 | 0.94 | 35.18(36.35) | 23.34(4.98) | 19.00(4.47) | 284.00(16.88) | 626.67(25.05) |
| *P. fluorescens* | 8.90 | 0.62 | 6.57 | 0.44 | 67.03(54.93) | 25.67(5.16) | 20.34(4.61) | 277.00(16.67) | 764.67(27.67) |
| Parthenium | 22.87 | 2.79 | 20.30 | 1.75 | 17.40(24.64) | 13.34(3.78) | 10.34(3.36) | 255.00(16.00) | 329.67(18.18) |
| Garlic | 19.84 | 2.25 | 17.54 | 1.47 | 22.59(28.36) | 18.34(4.39) | 14.34(3.91) | 269.34(16.44) | 525.34(22.94) |
| *T. harzianum* + parthenium | 15.80 | 1.87 | 15.27 | 1.25 | 14.81 (22.60) | 10.67(3.41) | 8.67(3.10) | 248.67(15.80) | 263.34(16.25) |
| *T. harzianum* + garlic | 25.50 | 3.51 | 21.94 | 2.35 | 11.48(19.76) | 6.67(2.76) | 3.67(2.15) | 238.67(15.48) | 163.67(12.83) |
| *P. fluorescens* + parthenium | 10.37 | 0.88 | 8.24 | 0.66 | 19.25(26.01) | 15.00(3.99) | 10.67(3.41) | 262(16.21) | 424.34(20.62) |
| *P. fluorescens* + garlic | 11.90 | 0.91 | 10.54 | 0.87 | 25.55(30.34) | 21.67(4.76) | 18.67(4.43) | 274.34(16.59) | 586.67(24.24) |
| Control | 6.24 | 0.43 | 4.70 | 0.37 | 71.11(57.46) | 29.34(5.50) | 23.34(4.93) | 298.67(17.31) | 874.34(29.58) |
| **SEm±** | **0.19** | **0.02** | **0.20** | **0.01** | **0.63** | **0.08** | **0.09** | **0.02** | **0.03** |
| **CD 5%** | **0.59** | **0.06** | **0.61** | **0.05** | **1.91** | **0.24** | **0.28** | **0.07** | **0.10** |

Average of three replications, PDI = Per cent disease incidence, *Figures given in parentheses are angular transformed, ** Figures given in parentheses are square root $\left(\sqrt{x+0.5}\right)$ transformed, Bio-agents = Soil application (@ 2.5kg/ha), Botanicals = Seed soaking (@ 10% for 30 min).

**Table 2 :** Effect of phyto-extracts and bio-agents on PO, PPO, PAL and Phenol concentration in fennel roots.

| Treatments | Concentration of enzymes and phenol | | | |
|---|---|---|---|---|
| | PO (µmol/min/g) | PPO (µmol/min/g) | PAL (µmol/min/g) | Phenol (mg/g) |
| *T. harzianum* | 0.621 | 0.042 | 0.212 | 1.910 |
| *P. fluorescens* | 0.593 | 0.040 | 0.182 | 1.826 |
| Parthenium | 0.677 | 0.076 | 0.442 | 2.080 |
| Garlic | 0.648 | 0.053 | 0.382 | 2.043 |
| *T. harzianum* + parthenium | 0.734 | 0.080 | 0.554 | 2.423 |
| *T. harzianum* + garlic | 0.788 | 0.094 | 0.646 | 3.110 |
| *P. fluorescens* + parthenium | 0.675 | 0.073 | 0.427 | 2.050 |
| *P. fluorescens* + garlic | 0.646 | 0.043 | 0.377 | 2.030 |
| Control | 0.423 | 0.027 | 0.117 | 1.513 |
| **SEm ±** | **0.001** | **0.000** | **0.000** | **0.009** |
| **CD 5%** | **0.003** | **0.000** | **0.001** | **0.029** |

Average of three replications, PDI= Per cent disease incidence, *Figures given in parentheses are angular transformed, ** Figures given in parentheses are square root () transformed, PO- Peroxidase, PPO- Polyphenol oxidase, PAL- Phenylalanine ammonia lyase, Bio-agents- Soil application (@ 2.5kg/ha), Botanicals- Seed soaking (@ 10% for 30 min)

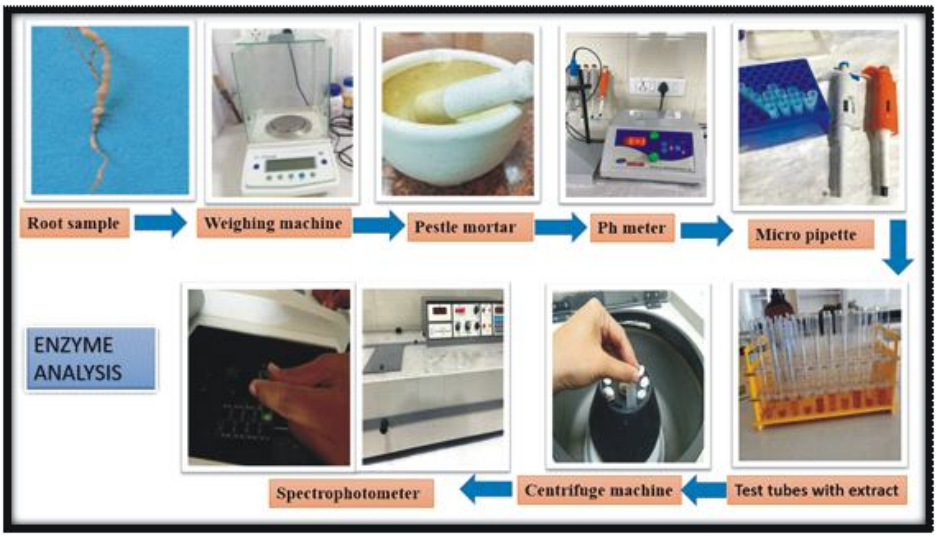


**Fig. 1 :** Enzyme analysis process.

neem cake with *Pasteuria penetrans* (Javed *et al*., 2008) in tomato and integration of *Pupurescillium lilacinus* with groundnut cake in eggplant (Ashraf *et al*., 2010) and castor cake and *P. penetrans* combination in chilli (Chaudhary *et al*., 2013) proved effective. Combination of neem cake, mustard cake, neem leaf extract and castor extract with *P. lilacinus* and *T. harzianum* against root nematode of brinjal and found most effective in managing the disease (Zareena *et al*., 2014). Dhayal *et al*. (2022) observed that neem extract @ 10 % concentration was found best to enhance PPO, PAL and phenol activity in tomato. Choudhary *et al*. (2023) found that neem cake (10%) was superior to enhance PPO, PAL and phenol activity.

## Conclusion

The use of bio-agents and botanicals is beneficial in reducing disease incidence and also in increasing the level of defense enzymes in plants as compared to diseased plants which also plays important role in reduced pathogen population and enhanced plant growth parameters. Soil application of *Trichoderma harzianum* (@ 2.5kg/ha) + seed soaking in garlic extract (10% for 30 min) was proved effective in enhancing plant biomass and in reducing the disease incidence. In conclusion, the use of the *T. harzianum* + garlic extract can be considered as a tool complementary for the commercial management of root rot of fennel in areas where this disease is gaining importance and becoming a limiting factor. Additionally, it can be used for managing pathogen resistance as well

as also provide opportunity for organic production of spices for consumers.

**Competing interests :** Authors have declared that no competing interest exist.

**Authors' contributions**

Methodology: 'Kiran Kumawat' conducted the original experiment, 'S. K. Goyal' and 'B. S. Chandrawat' wrote original draft manuscript, 'G. K. Mittal' and 'R. P. Ghasolia' done biochemical and statistical analysis, 'Lalita Lakhran' and 'B. L. Naga' reviewed and edited the MS and 'Dinesh Kumar Meena and Sushila Yadav' managed the literature.

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