Research Article

Effect of Two Nematicides on Nematodes Populations and Two Bacterial Populations Associated with Brinjal (*Solanum melongena* L.) Crop

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Abstract | The study involves evaluation of two nematicides, namely Turtob-F (a new nematicide) and carbofuran on 3 nematode populations and two bacteria associated with the rhizosphere of brinjal. The effect of the nematicides was observed on the population size of the selected nematodes and bacteria as well as the yield of brinjal crop. All three nematode populations were markedly reduced by the applied nematicides. The bacterial populations (*Pseudomonas* and *Klebsiella*) however, remained uninfluenced by the nematicides. The yield of brinjal was found to be significantly elevated ($p<0.05$).

Received | June 07, 2020; **Accepted** | June 21, 2021; **Published** | July 08, 2021
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Citation | Khan, A., Sheikh, S.A., Khanzada, K.A., Shaukat, S.S. and Akhtar, J. 2021. Effect of two nematicides on nematodes populations and two bacterial populations associated with brinjal (*Solanum melongena* L.) crop. *Pakistan Journal of Nematology*, 39(1): 59-64.
DOI | https://dx.doi.org/10.17582/journal.pjn/2021/39.1.59.64
Keywords | Brinjal, Nematodes, Bacteria, Nematicides, Karachi

Introduction

Eggplant, brinjal or aubergine is a plant species in the Nightshade family Solanaceae. It is grown worldwide and its edible fruit.

It can have bitter taste, with an slightly acidic quality, but it becomes tender when cooked and develops a rich complex flavor, salting may remove its bitterness. Eggplant is used in the cuisines of many countries especially in vegetarian cuisines (Anonymous, 2013). It grows in tropical and subtropical climates. Spacing between plants is usually 45 to 60 cm. In Pakistan, brinjal is used as a common vegetable in culinary due to its availability at reasonable price round the year. It is grown in all four provinces of Pakistan on small to large scale. The total area in the country under brinjal cultivation was 8575 hectares with a total annual production of 87585 tonnes during 2018-2019. Province wise brinjal cropped area and production was as follows: Punjab had the largest area under cultivation of brinjal which was more than 55% of the total area followed by Sindh (17.4 %), Balochistan (15.2 %) and KP (12.1%). The maximum contribution in the production of brinjal was of Punjab (68.36 %), followed by Balochistan (11.18 %), KP (10.51 %) and Sindh (9.93 %). Punjab province had relatively high share both in cropped area and production during 2018-2019 (Anonymous, 2020).

Brinjal is an important source of nutrition; raw eggplant is 92% water and 6% carbohydrates, 1% protein and has negligible fat. Minor changes in nutrient composition occur with environment of cultivation and season (José *et al.*, 2014).

Plant nematodes are considered as an important pest of brinjal plantation in major growing areas of

Pakistan (Shaukat *et al*., 2004; Zeerak *et al*., 2017; Ali *et al*., 2021). It causes severe root damage leading to stunting, chlorosis and drastic yield reduction. In tomato and eggplant 50-60% losses due to RKN was reported by Lamberti (1979) while 70-90% yield losses in tomato and brinjal due to nematodes was observed by Alam and Jairajpuri (1990). At present 18 nematode genera and 27 species have been reported from brinjal in Pakistan (Zarina and Shahina, 2012). The chemical nematicides have been used extensively for controlling nematodes associated with vegetables but reliance on these nematicides is associated with heavy cost, negative effect on human and animal health besides environment (Hemmati and Saeedizadeh, 2019).

The objective of the present work was (i) to determine effect of newly developed nematicide Turtob-F (a) nematicide containing 75% turmeric, 21% tobacco salt leaves and 4% captan a fungicide produced by PCSIR Laboratories Complex in collaboration with Crop Diseases Research Institute, PARC, Karachi) on population of plant nematodes (ii) to observe the effect of the amendments on yield (iii) to access their effect on two gram negative bacteria associated with rhizosphere of brinjal.

Materials and Methods

A survey was conducted during October 2020 to check initial population of nematodes in vegetable fields of Crop Diseases Research Institute, P.A.R.C., University of Karachi, Sindh, Pakistan. The rhizosphere soil was collected from a depth of 0-30 cm. Population density of these three plant nematodes as determined by a sieving and decantation and modified Baermann funnel technique (Southey, 1986). Five ml aliquots (15 replicates) of nematode suspension was used for nematode counts and values converted to number of nematodes per 200m^3 of soil sample. The population of *Helicotylenchus indicus* Siddiqi, 1963 was 54±10.5, *Hoplolaimus pararobustus* (Schuurmans and Teunissen, 1938) Sher, 1963 was 56 ± 18.8 and *Meloidogyne incognita* Kofoid and White, 1919 larvae was 210±4.2.

For preparation of treatments Tobacco sand leaves (*Nicotiana tobacum* L.) were dried at 58°C ± 2°C for 6 weeks, crushed into powder form and used. Carbofuran a chemical nematicide a.i. 44% Agricultural products group of FMC corporation, Philadelphia, PA, USA belonging to carbamate group of pesticide was used. The microplots each measuring 1m^2 containing sandy loam soil were tilled and amendmended with (i) Turtob-F (800 Kg/ha) (ii) Carbofuran at a rate of 15 Kg/ha. Unamended plots served as controls. All treatments were arranged in a randomized complete block design, each treatment was replicated four times. The initial population of nematodes was obtained by Baermann funnel technique. Ten days prior to applying amendments and 16 days prior to transplant seedling, observation on yield started after 12 weeks and four pickings were done within 6 weeks. Weeds present in the plots were regularly removed.

Isolation of selective plant benefitting bacteria from soil samples.

(i) *Pseudomonas* spp.: For the isolation of *Pseudomonas* spp. samples from different treatments Turtob-F, Carbofuran and control dilution upto 10^3 were collected in phosphate buffer (p^H 7.2).

Dilutions of each treatment were then inoculated in citramide medium (Oxoid, Germany) which is a *Pseudomonas* selective agar medium through pour plate technique. After inoculation in the selective medium plates were incubated for 24 h at 37°C.

Later the plates were checked for green pigmented selective colonies of *Pseudomonas* sp. Colonies were counted throuogh colony counter and results calculated and recorded.

(ii) *Klebsiella* spp.: Similar to *Pseudomonas* spp. dilution of treatments were prepared. The dilutions were then inoculated in EMB medium (Oxoid) which is a selective agar medium through pour plate technique. After inoculation in selective medium plates were then incubated for 24 hrs at 37°C. Later plates were checked for large mucoid pink purple *Klebsiella* spp. Colonies were counted by colony counter and the results recorded.

Data was subjected to analysis of variance (ANOVA). Duncan's multiple range test (LSD) were used as a post-hoc test in accordance with Zar (2008).

Results and Discussion

The population of *Helicotylenchus indicus* was significantly and effectively controlled by Turtob-F followed by Carbofuran ($p<0.05$).

The population of *Hoplolaimus pararobustus* was remarkably controlled by Carbofuran followed by Turtob-F ($p<0.05$).

Whereas the population of *Meloidogyne incognita* was effectively controlled by Carbofuran followed by Turtob-F ($p<0.05$).

The ANOVA (Table 1) showed that nematicides were highly significant ($p<0.01$) and the yield was significantly ($p<0.05$) elevated in treated plots (Table 2).

As far as both gram-ve bacteria (*Pseudomonas* spp. and *Klebsiella* spp.) their population was not affected by any of the treatments (Table 3).

Table 1: *ANOVA table of three different nematode species and treatments (nematicides).*

Source of variation	SS	df	MS	F	Significance
Populations	85977.72	2	42988.8	4.347	p<0.05
Treat. (Nematicides)	155918.38	2	77959.2	7.884	p<0.01
pop×treat.	82613.77	4	20653.4	2.008	n.s.
Error	266975.7	27	9887.9	–	–
Total	591485.63	35	–	–	–

L.S.D$_{.05}$ Nematode population = 83.29; L.S.D$_{.05}$ Treatments (Nematicides) = 83.29.

Table 2: *ANOVA table for yield.*

Source of variation	SS	df	MS	F	Significance
Treatments	22058.1	2	11029.03	6.26	p<0.05
	15850.9	9	1761.19	–	p<0.198
Total	37908.9	11	–	–	–

L.S.D$_{.05}$= 67.2

Table 3: *ANOVA for bacterial population in different treatments.*

Source of variation	SS	df	MS	F	p
Bacteria	164.3266	1	164.32	3.89	0.0641 n.s.
Treatments	150.55	2	75.27	1.78	0.1966 n.s.
Interaction Bacteria × treat.	124.18	2	62.09	1.47	0.2561 n.s.
Error	260.015	18	42.22	–	–
Total	119.005	23			

L.S.D$_{.05}$ Bacteria spp. = 5.57; L.S.D$_{.05}$ Treat. (Nematicide) = 6.82.

In the present experiment vast difference in treated and control plants was obvious in third week of transplantation (Figure 1). No significant change was observed in initial populations of *Pseudomonas* spp. and *Klebsiella* spp. at the end of the experiment (Figure 2).

Figure 1: *Difference in plant growth of control and treated brinjal plants.*

The plant nematodes are harmful to Agricultural crops. Severe quantitative and qualitative economic losses are attributed to their infestation around the world on a yearly basis. Excessive use of nematicides may lead to destruction of biodiversity. Many aquatic organisms, birds and animals are under threat to harmful pesticides for their survival. They have also threatened the survival of some rare species such as falcon, frogs, peregrine falcon, bald eagle, and osprey (Helfrich *et al*., 2009). Besides water, air and soil bodies have also been contaminated with these chemicals.

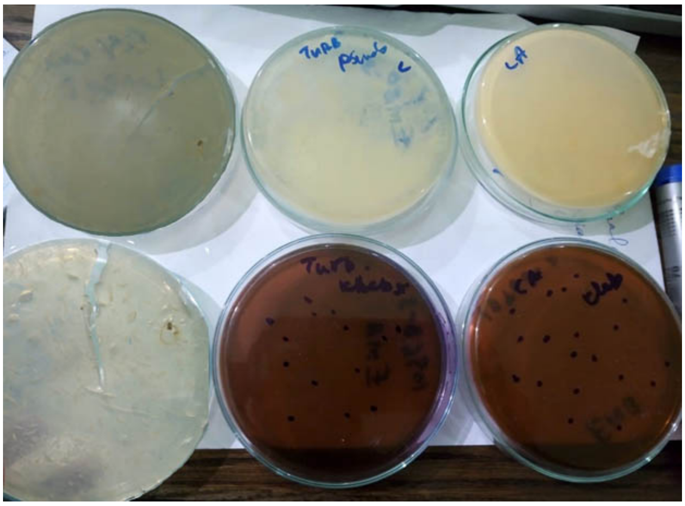

Figure 2: *Figure showing Pseudomonas spp. (first row) and Klebsiella spp. (second row) in control, Turtob-F and carbofuran.*

Thus, the need for alternative control which is eco-friendly has become imperative. Due to toxicities by chemical nematicides application of botanicals, rank high as compared to other methods owing to their environment safety. Almost 2400 plant species worldwide are known to have pesticidal properties (Atolani and Fabiyi, 2020).

In the present study a newly developed nematicide was used to control population of three plant nematodes and two gram-ve bacteria in the rhizosphere soil of brinjal along with its effect on the yield. The basic components of Turtob-F were turmeric powder and tobacco salt leaves besides a fungicide Captan was added to prevent growth of fungi and their spores.

Turmeric powder is about 60-70% carbohydrates, 6-13% water, 3-7% fat, 5-10% protein, 3-7% dietary minerals, 3-7% essential oils, 2-7% dietary fiber and 1-6% curcuminoids (Nelson *et al*., 2017).

Besides thirty-four essential oils are present in turmeric along with atlantone, germacrone, turmerone and zingiberene are major constituents (Braga *et al*., 2003) and has been used with great success to reduce nematode population (Neeraj *et al*., 2017). Tobacco salt leaves have alkaloid material, the best known is nicotine apart from this the other alkaloids are anabasine, nicotyrine, nornicotines, myosmine, nicotelline and isonicoteine. Tobacco leaves have antihelminthic effects (Budavari, 1989). Yu and Potter (2008) used nicotine *in vitro* against *Meloidogyne incognita* and *Heterodera schachtii* from strawberry and tobacco fields.

In the present study population of the three nematodes was significantly reduced along with yield was elevated but at the same time population of bacteria was not altered.

Khan *et al.* (2019) reported that leaves of *Phyllanthus amarus* plant may be used successfully for management of *Meloidogyne incognita* and could be a possible replacement of synthetic nematicides.

The aqueous and ethanol extracts of *Allium sativum* has been used to effective manage *Meloidogyne javanica* (Abbas *et al*., 2019). Previous report indicates that *Urtica urens* showed prominent resistance to plant nematodes such as *Aphelenchoides* sp.; *Meloidogyne incognita; Helicotylenchus* sp. and *Pratylenchus* sp. (Nassar, 2016). The investigation suggests that turmeric and tobacco can be successfully used to control nematodes associated with brinjal. Khan *et al.* (2021) reported control of *Tylenchorhynchus annulatus; Xiphinema* sp. and *Hoplolaimus pararobustus* associated with coriander in a pot experiment.

In the present trial the population of both *Pseudomonas* and *Klebsiella* which may be beneficial to the plants was not significantly reduced. However more detail study is required to identify and evaluate plants having nematicidal properties which could replace some of the existing toxic chemical nematicides.

Conclusions and Recommendations

The information derived from this experiment suggests that Turtob-F can be used for the management of *Helicotylenchus indicus, Hoplolaimus pararobustus* and *Meloidogyne incognita* and in future may be used as a replacement for chemical nematicides with no effect on beneficial bacterial flora.

Novelty Statement

The study involves evaluation of two nematicides, namely Turtob-F (a new nematicide) and carbofuran on 3 nematode populations and two bacteria associated with the rhizosphere of brinjal. The effect of the nematicides was observed on the population size of the selected nematodes and bacteria as well as the yield of brinjal crop. All three nematode populations were markedly reduced by the applied nematicides. The bacterial populations (Pseudomonas and Klebsiella) however, remained uninfluenced by the nematicides. The yield of brinjal was found to be significantly elevated ($p<0.05$).

Author's Contribution

AK and SAS: Planned the experiment and wrote the manuscript.
KAK and JA: Helped in field trial and collection of data.
SSS: Did statistical analysis.

Conflict of interest
The authors have declared no conflict of interest.

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