LCCN 85-931448

ISSN: 1023-1072 (Print), ISSN: 2663-7863 (Online)
https://doi.org/10.47432

PAKISTAN JOURNAL OF AGRICULTURE AGRICULTURAL ENGINEERING AND VETERINARY SCIENCES

An Official International Biannual Publication of Sindh Agriculture University Tandojam, Pakistan

Volume 39 (1) June, 2023

SINDH AGRICULTURE UNIVERSITY TANDOJAM

http://pjaaevs.sau.edu.pk/index.php/ojs

Pak. J. Agri., Agril. Engg., Vet. Sci., 2023, 39 (1): 32-36
ISSN: 1023-1072 (Print), ISSN: 2663-7863 (Online)
https://doi.org/10.47432/2023.39.1.6

EFFECT OF PLANT BASED NEMATICIDE ON POPULATION OF PARASITIC NEMATODES AND BACTERIA ASSOCIATED WITH OKRA (*ABELMOSCHUS ESCULENTUS* L. MOENCH)

A. Khan[1*], S. A. Shaikh[2], K. A. Khanzada[1], S. S. Shaukat[3] and K. Siddiqui[2]

[1]CDRI, Pakistan Agricultural Research Council, University of Karachi, Pakistan
[2]PCSIR Laboratories complex, Karachi, Pakistan
[3]Institute of Environmental Studies, University of Karachi, Pakistan

ABSTRACT

Rapid declines in nematode populations has been observed by the use of organic amendments in soil when decomposing plant based material release toxic compounds, while long term effects include increase in the yield and have no toxic effect on soil bacteria unlike the chemicals. In the present experiment Turtob-F was used to control population of three plant nematodes and to observe its effect on yield and population of *Klebsiella* sp. in soil. The population of all three nematode namely *Meloidogyne incognita, Helicotylenchus pseudorobustus* and *Tylenchorhynchus indicus* were remarkably decreased (P<0.001) by Turtob-F. The yield of okra was significantly increased (P<0.01) by the application of Turtob-F, whereas the population of bacterial sp. *Klebsiella* remains unchanged during the experiment.

Keywords: bacteria, Karachi, nematodes, okra, turtob-F

INTRODUCTION

Okra *Abelmoschus esculentus* L. Moench belongs to the family Malvaceae which originated in Africa and Asia (Thomson and Kelly, 1979) it is an important crop in Pakistan, as it is highly nutritious mostly eaten while pod is green, immature and tender (Olabiyi and Oladeji, 2010). Due to rampant use of chemical nematicides which have harmful effects on environmental side and health, there is a strong need for alternative pest control methods. Important source of biorational compound occurring in plants as products of secondary metabolites (D' Addabbo *et al.,* 2014).

Physical and chemical properties of soil are substantially improved by the use of plant materials which ultimately control plant nematodes, do not affect population of *Klebsiella* sp. and increase crop yield. Rapid decrease in nematode antagonists in long term are caused by organic plant based amendments resulting in better plant growth (McSorley, 2011).

Plant parasitic nematodes present in rhizosphere soil are subject to infection by bacteria and fungi, which creates capability of using soil microorganisms to control nematodes (Jatala, 1986). Bacteria are the most abundant microorganisms in soil (Tian *et al.,* 2007). They attack plant nematodes by a variety of modes such as parasitizing, producing toxins or enzymes interfering with nematode plant host recognition, inducing systematic resistance of plants, promoting plant health, competing for nutrients (Siddiqui and Mahmood, 1999). *M. incognita* are endoparasites whose adult female grow inside the roots leading to substantial losses in yield due to presence of giant cells within host roots (Vilela *et al.,* 2021). *H. pseudorobustus* is an ectoparasite which causes reduction in root system leading to unhealthy plants (O'Bannon and Inserra, 1989). *T. indicus* is an ectoparasite which cause stunting of root system and in some cases yellowing of foliage and defoliation and are mildly pathogenic (O'Bannon *et al.,* 1991).

At present the use of plant based nematicides are becoming more popular as an additive instead of chemical nematicides for improving crop yield, controlling nematodes and not harming beneficial bacteria. In this regard use of Turtob-F a nematicide containing 75% turmeric, 21% tobacco salt leaves and 4% Captan a fungicide is being used. Now we

*Corresponding author: aly.khan@hotmail.com

extend this investigation by studying (i) effect on nematodes (ii) effect on yield and (iii) to assess their effect on gram-ve bacteria *Klebsiella* sp. associated with okra in a field trial.

MATERIALS AND METHODS

Okra *Abelmoschus esculentus* var. Green Golden were sown on 1 October 2021 initial population of nematodes were checked one week prior to plantation in vegetable fields of CDRI, Pakistan Agricultural Research Council, University of Karachi, Sindh, Pakistan. The rhizosphere soil was collected from a depth 0-25cm with the aid of hand shovel. Samples were stored in polythene bags. All samples were marked with a permanent marker on the host, date and locality. Population density of the three plant nematodes species *M. incognita, H. pseudorobustus* and *T. indicus* were determined by 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 *Meloidogyne incognita* Kofoid and White, 1919 was 91.3±5.60; the population of *Helicotylenchus pseudorobustus* (Steiner, 1941) Golden, 1956 was 64.33±18.85 and *Tylenchorhynchus indicus* Siddiqi, 1961 was 41.0±15.63. Total crop period was 11 weeks. Microplots 1m^2 containing sandy soil were tilled and amended with (i) Turtob-F (800 kg/ha), this new nematicide has been produced by C.D.R.I, Pakistan Agricultural Research Council, University of Karachi in collaboration with PCSIR Laboratories' Complex, Karachi (ii) carbofuran a chemical nematicide (i). 44%, Agricultural products group of FMC corporation, Philadelphia, PA, USA belonging to carbamate group of pesticide was used (15 kg/ha). Plots without treatment were kept as control.

In order to prepare Turtob-F, turmeric powder purchased from local market tobacco sand leaves were added which were dried at 56±2ºC for 6 weeks, later sand leaves were homogenized to coarse particles using blender and captan a fungicide registered in the United States on March 8, 1949 which is found in formulation with a number of other pesticides was added to it. The three were used in the ratio (75:21:4). For bacteria soil was initially diluted and samples were plated on selective medium EMB medium (oxoid) for isolation of *Klebsiella* sp. (Figure 1). Initially string test was done (Figure 2) and later citramide medium test was performed at 35ºC aerobically for 4-6 days (Figure 3). There were three picking of okra which were started at 7 weeks. All treatments were arranged in a randomized complete block design, each treatment was replicated three times. Weeds present in the plots were regularly removed. At the time of final harvest population of nematodes and *Klebsiella* sp. along with yield was recorded. Watering was done twice a week except at 4 weeks due to sudden heat (38±2ºC) had to be watered daily (Figure 4). The data was subjected to ANOVA (analysis of variance) or FANOVA (factorial analysis of variance) depending on the factors considered in accordance with Zar (2008).

RESULTS

Analysis of variance (ANOVA) was used as a statistical technique to check if means of two or more groups are different significantly from each other.

Effect on population of nematode

Turtob-F significantly decreased the populations of the three nematodes namely *Meloidogyne incognita* Kofoid and White, 1919; *Helicotylenchus pseudorobustus* (Steiner, 1941) Golden, 1956 and *Tylenchorhynchus indicus* Siddiqi, 1961 (P<0.001). The interaction between treatment x nematode species was also disclosed as significant (P<0.01) (Table 1). Population of *Klebsiella* sp. remained unchanged (non-significant) throughout the experiment by Turtob-F (Table 2).

Yield of okra crop

The effect of Turtob-F on yield was seen to be highly significant (P<0.001). In the control the yield was about 12% lesser than that of Turtob-F. The first picking of okra was highest while the third was the least (Figure 5; Table 3).

Table 1. ANOVA for different treatments on population of three plant nematodes namely *M. incognita, H. pseudorobustus* and *T. indicus*

Source	SS	df	MS	F
Tre	18042.84	02	11.92	0.0001
Sp	8303.64	02	05.50	0.0002
Tr X S	6859.42	04	02.20	0.0001
Error	60332.31	36	-	-
Total	60332.31	44	-	-

LSD Treatments = 20.328 LSD Sp. = 20.328

Table 2. ANOVA for Okra *Abelmoschus esculentus* L. Moench variety Green Golden yield (3 pickings)

Source	SS	df	MS	F	
Dates	25163.18	02	12581.51	45.84	0.0001
Tr	95227.0	02	47613.59	1734	0.0001
DXTr	29423.70	04	7355.92	26.80	0.0001
Error	4940	18	274.44	-	-
Total	154754.03	26	-	-	-

LSD Tre = 16.40 LSD Sp. = 16.40

Table 3. ANOVA for population of Gram –ve bacteria (*Klebsiella* sp.)

Source	SS	df	MS	F
Before/After	10.88	01	10.88	0.136 ns
Treat	609.77	02	304.83	3.816 ns
Interaction B/A x Tr	56.14	02	28.22	0.353 ns
Error	958.66	12	79.88	-
Total	1635.77	17	-	-

LSD Before/After = 9.180 LSD Treatments = 11.243

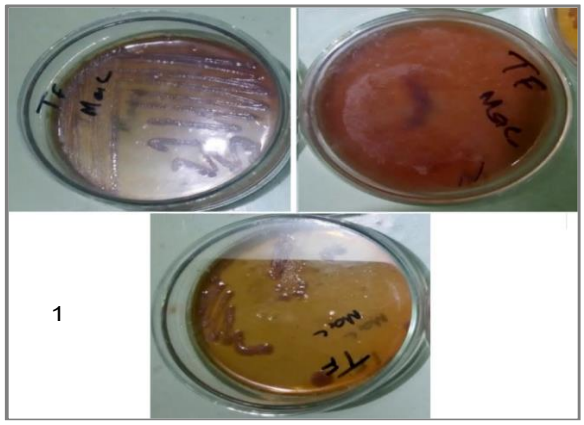

Figure 1. *Klebsiella* sp. on EMB medium (oxoid)

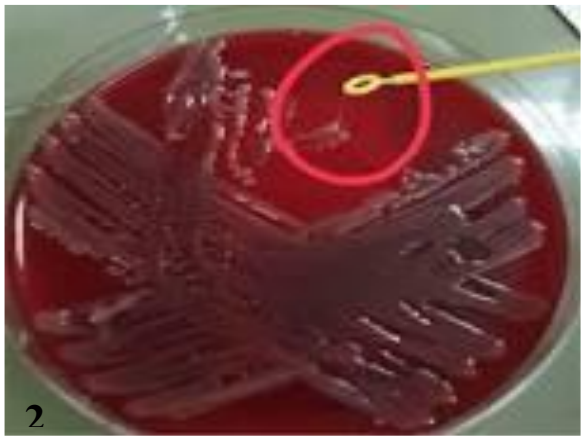

Figure 2. String test

Figure 3. Citramide medium test for confirmation of *Klebsiella* sp

Figure 4. Okra in third week when daily watering was done

Figure 5. Third picking of okra

DISCUSSION

The population of all the three nematodes namely *M. incognita, H. pseudorobustus* and *T. indicus* was significantly reduced by Turtob-F resulting in enhancement of yield while the population of *Klebsiella* sp. remained unaltered. Chitwood (2002) discovered a number of phyto-chemicals antagonistic towards nematodes. Higher plants yielded broad spectrum of active compounds which includes polyacetylenes, polythienyls, isothiocyanates, cyanogenic glycosides, glucosinolates, lipids, alkoloids, terpenoids, quassinoids, sesquilepenoids, triterpenoids, simple and complex phenolics and several other classes. These natural products act against mammalian parasites and can serve as useful source of compounds which could be examined against plant parasitic nematodes. The use of chemical nematicides alters the population of beneficial microorganisms (Diez, 2010; Fang *et al.,* 2014).

Bhardwaj *et al.* (2017) suggested that bacteria *Klebsiella* sp. has solubilization of phosphate, phytohormone production and good germination properties in vivo cereal crops experiment. Jha *et al.* (2021) suggested that *Klebsiella pneumoniae* PVN-1 isolated from effluent treatments plant (ETP) found inorganic phosphate solubilizing potential (528.5 mg/L). Along with, the genetic potential for complete

catechol, benzoate and phenylacetate degradation with stress response and heavy metals (Zn, Ca, Co, Ni) resistance was identified in *K. pneumoniae* PVN-1. Khan *et al.* (2021, 2021a) have successfully used Turtob-F to control nematodes associated with coriander. The population of *Tylenchorhynchus annulatus* was effectively controlled by carbofuran followed by Turtob-F (p at the most 0.05) @ 9g/kg soil in pot experiment while nematode *Helicotylenchus indicus* associated with brinjal was effectively controlled by Turtob-F as compared by carbofuran while the population of *Pseudomonas* spp. and *Klebsiella* spp. remained unchanged by Turtob-F.

Sapre *et al.* (2018) in an experiment on salt tolerant PGPR strain 1G3 on growth of oat seedlings which were under salinity stress found that PGPR strain 1G3 (*Klebsiella* sp.) showed better shoot dry weight, length of root and root dry weight significantly higher in PGPR inoculated plants in comparison to positive control plants under non stress condition. Hayat *et al.* (2010) found that bacteria belonging to *Klebsiella* group attach to the root and efficiently colonize root surface which results in sustainable growth promotion of plant. Iniguez *et al.* (2004) suggested that wheat inoculated with nitrogen fixing bacterium *K. pneumoniae* 342 (Kp 342) relieved nitrogen (N) deficiency symptoms and increased total N and N concentration in the plant. Triplett *et al.* (2008) reported that *K. pneumoniae* which is a biological inoculant enhanced the growth of cereal grasses. Therefore, use of plant based nematicides which would be toxic to plant nematodes but does not affect population of plant-growth-promoting bacteria is highly recommended.

CONCLUSION

The plant material nematicides as amendments constitute a valid alternate to toxic chemical nematicides in reducing plant parasitic nematodes at the same time not affecting population of *Klebsiella* sp. and enhancing crop yield. The integration of data knowledge in the field after the treatment with plant materials together with understanding bacterial population and their action against nematodes at molecular level may provide basis for bettering pathogenic activity of potential biocontrol strains as well.

AUTHOR'S CONTRIBUTION

A. Khan: Conducted the trial and wrote manuscript.
S. A. Shaikh: Conducted experiment.
K. A. Khanzada: Conducted experiment.
S. S. Shaukat: Dod statistical analysis.
K. Siddiqui: Provide literature.

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(Received: September 30, 2022; Accepted: May 17, 2023)