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Publisher of open access books and journals
PJMLS
Print ISSN: 2707-4471. Online ISSN: 2707-448X

Research Article	Pak-Euro Journal of Medical and Life Sciences
DOI: 10.31580/pjmls.v5i2.2417	Copyright © All rights are reserved by Corresponding Author
Vol. 5 No.2, 2022: pp. 419-428	
www.readersinsight.net/pjmls	
Submission: January 31, 2022	**Revised:** May 22, 2022 **Accepted:** June 20, 2022

IDENTIFICATION AND MANAGEMENT OF PARASITIC NEMATODES ASSOCIATED WITH MULBERRY IN MANSEHRA, KP, PAKISTAN

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Abstract

Parasitic nematodes are destructive parasites of crop plants and cause enormous damage to them. The earliest signs of nematodes attack are the inactiveness of growth as infestation of nematodes is a slow process. The survey was held to evaluate the percentage of the plant-parasitic nematodes that related to mulberry in various localities in the District Mansehra. For this purpose Mansehra, Balakot and Oghi were selected. From these different areas soil samples were collected and processed by using Baermann funnel technique to examine and calculate the percentage of saprophytic and parasitic nematode populations. From the results it had shown that samples from Mansehra and Balakot had more parasitic nematodes than saprophytic while samples from Oghi were found saprophytic. Tehsil Mansehra showed 65% whereas tehsil Balakot showed 35% population of parasitic nematodes. From Mansehra and Balakot, Helicotylenchus dihystera was found in profusion. For the management of parasitic nematodes inorganic amendment (Carbofuran) and organic amendments (Poultry manure) were used in 250 gram/tree and 8kg/ tree respectively. Samples and subsamples from both treated and untreated trees showed that Carbofuran exterminated the nematode population entirely whereas the population of parasitic nematodes decreased by the implementation of Poultry manure to a notable point. Histograms analysis and ANOVA was done. ANOVA showed that p value for both Carbofuran and Poultry manure impact in Mansehra and Balakot was 0.0001 and therefore was found significant. Thus both were found effective however Poultry manure is more eco-friendly in nature.

Keywords: *Balakot, Carbofuran, Histogram, Mansehra, Mulberry, Nematode, Plant parasitic, Poultry manure, Saprophytic*

INTRODUCTION

Mulberry is popular and most extensively distributed all over the world. Mulberry is edible and tastiest fruit and used to eat in both fresh and dry form. In mulberry there are great varieties that are different in physical shape, size, color and taste. Mulberry fruit has distinctive nutritional values. Pakistan is positioned in South Asia in the north western part. In Pakistan mulberry is extensively grown in northern areas and considerably distributed at high altitudes in the Hindu Kush Himalayan region and in colder regions such as Balochistan, Azad jammu and Kashmir, Chitral, Quetta (1). Mulberry plants are distributed

in various regions in District Mansehra, KPK, Pakistan. For the growth of mulberry the conditions of climate are very appropriate. The extremity of destruction and attack on mulberry plants by nematodes mainly depends on the wide variety of climate, topographical and soil conditions of the different areas (2). Nematodes are cosmopolitan, with an extensive range of hosts as well as related to other pests and also pathogens making the detection of the disease unclear (3). A serious loss to a vast diversity of crop plants is caused by parasitic nematodes economically. Plant parasitic nematodes are the causative agents and parasitize economically many important crops and plants including mulberry. Different research workers from all around the world had done their work on plant parasitic nematodes related to mulberry. They also recognized different parasitic nematodes related to the mulberry that causes severe damage as a consequence of loss in product yield (4).

It is significant to manage the parasitic nematodes because it reduces the number of parasitic nematodes to stage below the damage threshold preferably than destruction. Two types of amendments i.e. organic and inorganic amendments are used all over the world. In this study inorganic amendment (carbofuran, a nematicide) whereas organic amendment (poultry manure) were used against plant parasitic nematodes in Mansehra.

MATERIALS AND METHODS

Several different areas of District Mansehra were studied for mulberry trees. From the different regions of Mansehra, Balakot and Oghi, soil samples were collected from the mulberry trees. Samples of soil had been taken out from rhizosperes through digging a gap with the help of spade or trowel at the depth of 5 cm to 60 cm close to the root base relying on the size of tree trunk. Almost 350 grams of soil samples were taken and placed in the air tight plastic bags to keep the sample moist. And mentioned a name of host, location along with date labeled on it with the help of marker. It is very important to keep soil samples cool at the optimal temperature. From the infected plant the collected soil samples were processed through a modified Baermann funnel technique (5), in a laboratory (6). After 24 hours, Underneath Watson Barnet stereoscopic microscope at 4X power each soil sample per 200 ml of soil was observed for the population of plant-parasitic nematodes. In various areas of District Mansehra, to find out the parasitic nematode population, quantitative analysis was done.

For the purpose of management, carbofuran (nematicide) and poultry manure were implemented all around the nematode affected mulberry tree. Implementation of carbofuran was at the ratio of 250gm/ tree whereas 8kg/ tree of poultry manure was applied. The amendments were implemented in the soil by combining with the help of spade or trowel around the trunk of the selected tree. Watering was usually done and kept away from any agricultural spray or any type of pesticides. Soil samples along with subsamples of soil were collected after 3 Month, 6 Month and 12 Month of implementation and processed by using Bearmann funnel technique. Quantitatively the population of parasitic nematodes was analyzed under stereoscopic microscopy. Keep untreated trees for the comparison with the treated ones. Subjected the data collected to histogram analysis and ANOVA.

STATISTICAL ANALYSIS

One way ANOVA and histogram analysis were statistically done.

RESULTS

It was shown in the quantitative analysis that the population of nematodes consists of both saprophytic as well as parasitic species. Several areas of district Mansehra were entirely saprophytic but in some areas both saprophytic as well as parasitic nematode species were shown. Parasitic nematodes consist of various different species that are harmful to plant health. *Helicotylenchus* species that are fungal feeders in nature were found to be the most common species of plant parasitic nematodes. In Table I the absence or presence of parasitic nematodes is shown from the different areas in Mansehra. The positive (+) symbol

shows the presence and the negative (-) shows the absence of the parasitic nematodes. Localities we studied from the tehsil oghi were found saprophytic.

Table I. Presence (+)/absence (-) species of nematodes in different areas of Mansehra District

Localities	*Helicotylenchus dihystera*	*Pratylenchus Spp.*	*Helicotylenchus indicus*	*Tylenchorhynchus swatiensis*	*Helicotylenchus larvae*	*Paratylenchus projectus*
Datta	+	+	+	-	+	-
Ghazikot	+	+	+	-	-	-
Dhodial	-	-	+	-	+	-
Behrkund	+	-	-	-	+	-
Balakot	+	+	-	-	+	-
Satbani	+	-	+	-	-	+
Shinkiyari	+	-		-	+	+
Baffa	-	-	+	-	+	-
Mahandri	+	-	+	+	-	-
Garhi Habibullah	+	+	-	-	+	-
Kaghan	-	-	+	-	-	-

In different areas of Tehsil Mansehra and Balakot, the comparison and conclusion of parasitic nematode population indicated that; in Mansehra 65% and in Balakot 35% population was nematode (Fig. 1). Statistically, data was plotted in histograms showing the nematode population of both saprophytic and parasitic in different areas of Mansehra, Balakot and Oghi. One-way AVOVA of the data showed that *p* value was significant (p=<0.0001).

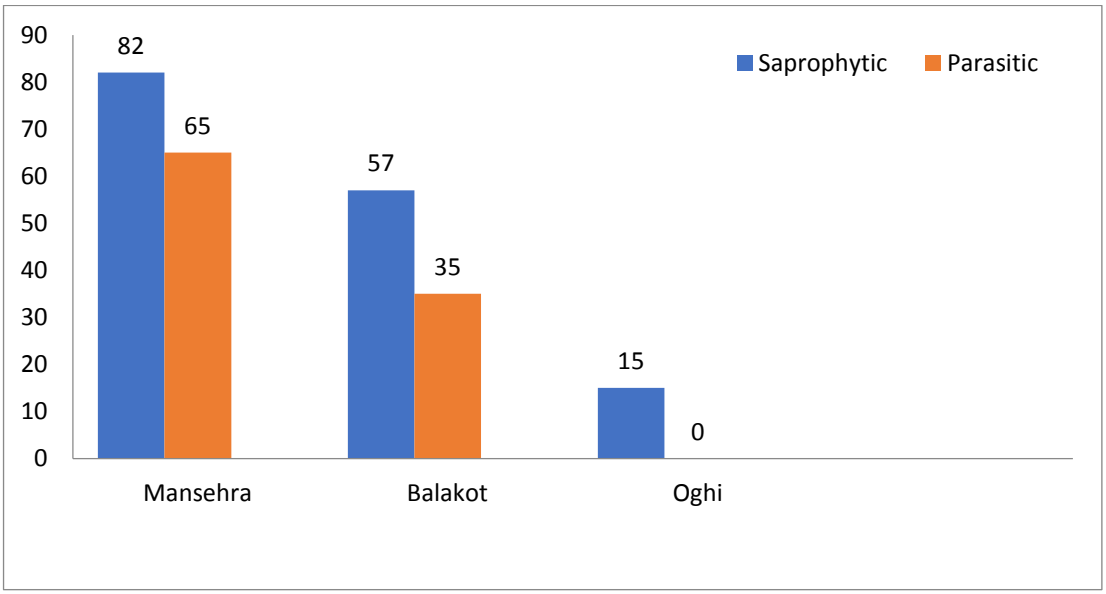

Fig. 1. Histogram bars showing the population of plant parasitic and saprophytic nematodes in different areas

Plotting the management data that were collected in graphs of histograms showed that implementation of both carbofuran (nematicide) and poultry manure (organic fertilizer) to manage the parasitic nematodes related to mulberry trees had shown optimistic results very clearly. Carbofuran and poultry manure both declined the population of parasitic nematodes related to mulberry trees in Mansehra and Balakot. In the histogram, graphs compared the data of treated trees with untreated trees (Fig. 2).

Carbofuran is an inorganic amendment i.e. a nematicide was commercially prepared for the management of parasitic nematodes related to different crops and plants and available in market. It was shown in histogram graphs that use of carbofuran declined the nematode population to a considerable extent in Mansehra and Balakot was very clear (Fig. 3).

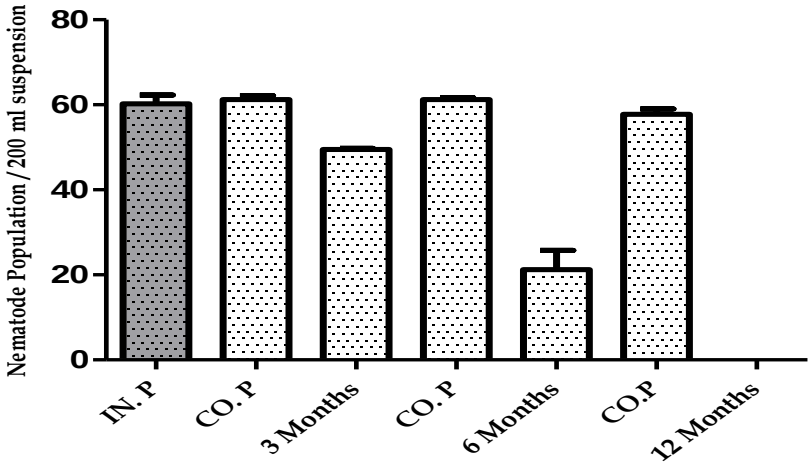

Fig. 2. The carbofuran (CF) effect on the population of parasitic nematodes is shown in histogram in Mansehra (IN.P=Initial population, CO.P= Controlled population)

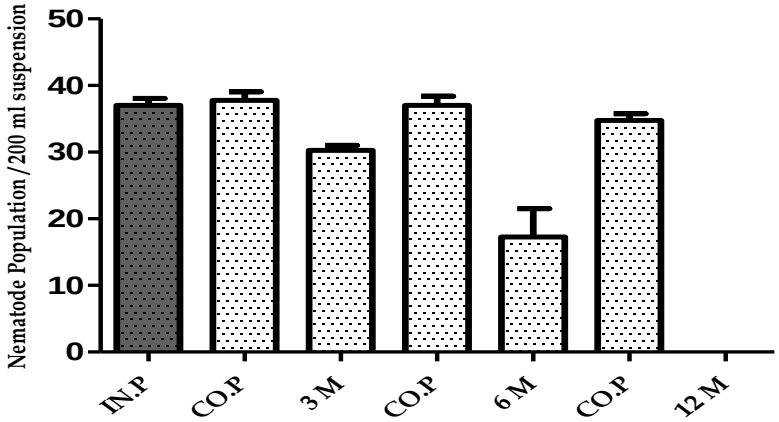

Fig. 3. The carbofuran (CF) effect on the population of parasitic nematodes is shown in histogram in Balakot (IN.P=Initial population, CO.P= Controlled population, M= Month)

Collected data for observation of the poultry manure effect on the population density of parasitic nematodes in histogram analysis shows that poultry manure has a vital role in decreasing the plant parasitic nematodes that infect the host plant. Therefore, reduction of parasitic nematode population improves the fertility of soil; enhances the health of trees as well as fruit. Poultry manure is organic in nature, is a compost of poultry and also easily available from poultry farms. It was shown in the data that the implementation of poultry manure declined the nematode population considerably. So it managed the population of parasitic nematodes in district Mansehra and Balakot also (Fig. 4 and Fig. 5).

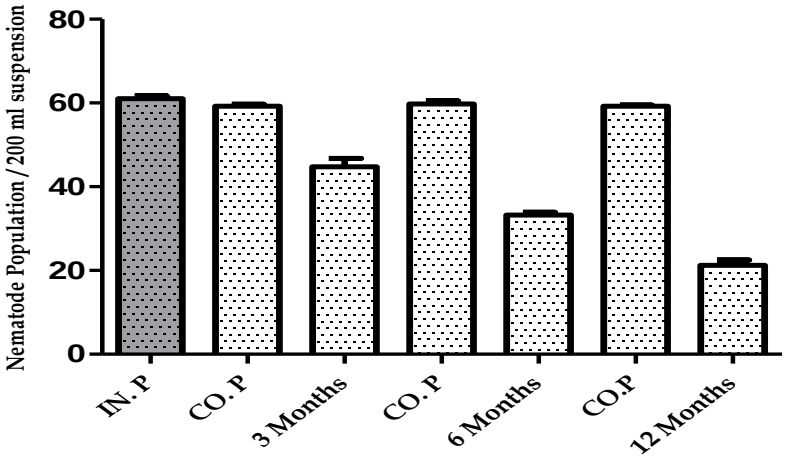

Fig. 4. The poultry manure (PM) effect on the population of parasitic nematodes is shown in histogram in Mansehra

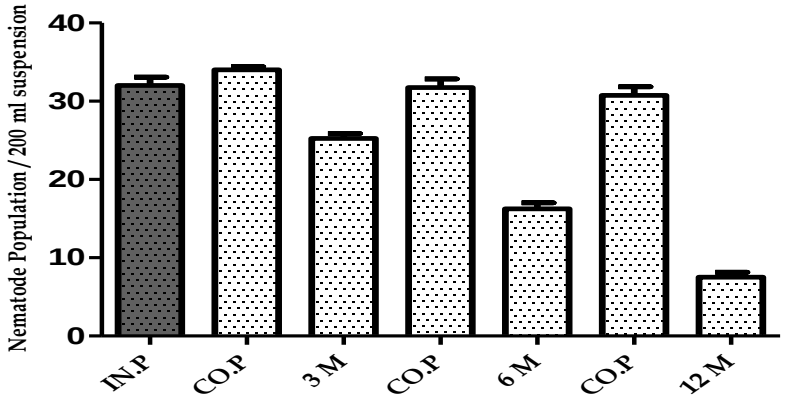

Fig. 5. The poultry manure (PM) effect on the population of parasitic nematodes is shown in histogram in Balakot (IN.P=Initial population, CO.P= Controlled population, M= Month**)**

DISCUSSION

In different study areas combined culture of parasitic nematodes were found that were damaging the trees and the symptoms of the attack by nematodes results chlorosis and wilting of leaves, retarded plant growth, less yield production, root knots, root lesions and tuber galls.

The isolated and identified parasitic nematodes belonging to the different families from different localities of district Mansehra were *Helicotylenchus dihystera, Pratylenchus spp., Helicotylenchus indicus, Helicotylenchus* larvae, *Paratylenchus projectus,* and *Tylenchorhynchus swatiensis.Paratylenchus projectus* nematodes also known as pin nematodes and are belong to the family Tylenchulidae (7). *Pratylenchus spp.* are called root lesion nematodes as well as are members of the family Pratylenchidae. *Helicotylenchus indicus, Helicotylenchus* larvae as well as *Helicotylenchus dihystera* all are fungal feeders and belong to the Haplolaimidae family. *Tylenchorhynchus swatiensis* (8) are known as stunt nematodes and are members of Belonolaimidae family.

Pratylenchus spp. are endo-parasitic root lesion nematodes and economically are very important nematodes. Into the plant's rhizospheres, these species of parasitic nematodes form necrotic lesions and cause severe damage to the host plant. These parasitic nematodes migrate and may possibly cause serious damage to the root cortex if their population density is high. Death of cortical cells take place due to which formation of cavities occur as well as make the host plant prone to fungal and also bacterial pathogens which puncture the plant through abrasion and damaged areas and cause secondary infections in the host plant . *Pratylenchus* spp. identified from the locaties of Gazikot, Shinkiyari, Balakot and Datta due to which mulberry plants show signs and indications of infection over there.

Paratylenchus spp. are also recognized as pin nematodes and are the smallest parasitic nematodes which assault plants. Pin nematodes are migratory ectoparasites and simply detect and travel towards the plant roots. Lateral roots emerge and are preferred to the main roots, as these are younger as a result small stylet of nematodes easily penetrated. All feeding stages of pin nematodes nourish through stylet that insert into epidermal cells, often close to the base of root hairs and occasionally on the root hairs. Pin nematodes might be found highly in numbers on numerous different plants. These nematodes attacking plants can turn into stunted with severe chlorosis (yellowing of leaves) and shallow localized lesions took place. The prolonged feeding of many individuals on the same root may lessen or end the rate of growth and development of lateral roots, nutrients may decrease and as a result energy that can be stored in the rhizome of the plant, dropping the health and vigor of the plants potentially. *Paratylenchus projectus* spp identified from the locality of Shinkiyari and Satbani.

Tylenchorhynchus swatiensis are stunt nematodes and this species is first recorded and illustrated from the specimens on *Prunus ameniaca* from Swat, Pakistan. This species may be prominent from all of the other species of the genus *Tylenchorhynchus* mainly on the basis of a combination of the distinctive, smaller body length, smaller length of stylet, completely set off cephalic region, presence of single epiptygma and

spicules and smaller gubernaculums. These are soil dwelling stunt nematodes, ectoparasitic nourish on the surface of roots and cause stress and diseases mainly stunted growth in plants, roots shrunken, short and sparse. *Tylenchorhynchus swatiensis* reported from the locality of Mahandri.

Kumari and Sujathamma (4) stated that among different factors, diseases are one of the main factors which affect productivity of mulberry. Root knot disease has adversely affected mulberry. They held out study to know the impact of root-knot nematode i.e. *Meloidogyne incognita*, Chitwood against the growth along with development related to different varieties of mulberry.

In West Bengal Tridevi *et al.* (2008) reported that mulberry plants are the hosts of the pathogens of various parasitic diseases. Three fungal foliar diseases, like Powdery mildew, Leaf rust and Leaf spot, one fungal Root rot, one nematode infected Root knot (in several limited areas of West Bengal) and one bacterial Leaf blight are common in West Bengal. Every year these parasitic diseases may cross serious economic threshold levels and cause an average of 25-30% crop loss.

Chanu and Meitei (2016) described the extremity of destruction and attack on mulberry plants by nematodes mainly depends on the wide variety of climate, topographical and soil conditions of the different areas (2). Narasimhamurthy *et al.* (2011) described that an attempt taken to control *Meloidogyne incognita* damaging mulberry plants by applying indigenous (Trichoderma viride, 2×10^6 cfu/g), commercial biological agents (Trichoderma viride, 2×10^6 cfu/g) and (Psuedomonas fluorescens, 1×10^8 cfu/g) under conditions of field. In inhibiting the population of nematodes all the biological agents noticed were effectual and also enhancing plant growth and yield production (8).

Collange *et al.* (2015) described those organic amendments such as poultry manure and cow dung, crop residues i.e. green manures, town wastes or waste material of industries i.e. oil seed cakes used for the control of parasitic nematodes (10). A few of them were used as mulches on the top layer of the soil while other residues were included into the soil like leaves of neem (i.e. *Azadirachta indica*) may be applied in soil by including the leaves as green manure or used as an extract for biological control of nematodes. Organic amendments enhance the soil capacity to control water and nutrients, which enhance plant vigor and as a result improves the plant tolerance to parasitic nematodes as they stimulate activities of microbes in the soil (containing antagonists).

Seetha *et al.* (2010) in their study carried out to control and manage the soil borne fungal pathogens i.e. *Altemaria tenuissima* and *Fusarium solani* (Mart.) Sacc *(7).* The causative pathogens of die-back and root rot diseases on mulberry stem cuttings planted nurseries of mulberry and also in established mulberry plantations. Under in vitro conditions, the extracts of ten plants with 10% concentration other than *Lantana camara* (undiluted) were tested through four bio-fungicides and poisoned food technique were also screened by dual culture method. The maximum inhibition showed by the plant extract of *Prosopis juliflora* on the mycelial growth (80.0% over *F. solani* and 81.2% over *A. tenuissima*) followed by *L. camara* (68.9% over *F. solani* and 66.7% over *A. tenuissima*). Under in vivo conditions along with the popular chemical Mancozeb, the adversaries (*T. viride* and *P. fluorescens*) and the plant extracts (*L. camara and P. juliflora*) were tested against both the pathogenic fungi. All the examined bio-fungicides and plant products showed inhibitory effects on both fungi.

Hassan *et al.* (2009) reported that poultry manure is more effectual than urea and super phosphate in management of parasitic nematodes such as *Tylenchus mirus, Helicotylenchus, Ditylenchus* sp. and *Pratylenchus scribneri* (11).

Shaukat *et al.* (2004) demonstrated that as the biocontrol agents, aqueous extracts of four species of weed i.e. *Amaranthus viridis* (L.), *Blumea obliqua* (L.) Druce, *Conyza Canadensis* (L.) Cronquist and *Eclipta prostrate* (L.) used to control the parasitic nematodes (12). These weed species caused considerable mortality rate ($P<0.05$) of the plant parasitic nematodes i.e. *Tylenchulus semipenetrans, Meloidogyne incognita* and *Xiphineyza americanum*. The weed species influence a differential impact on a different nematode species. For instance *A. viridis* caused most of the mortality of *X. americanum* while greater mortality of the juvenile stage of *T. semipenetrans* and *M. incognita* induced by *C. canadensis*. Whereas *B. obliqua* was least effective on parasitic nematodes.

Govindaiah worked on fumigant durofume (methyl bromide and ethylene dibromide) that was tested as a preplanting treatment against root knot disease and weeds in mulberry plants (13, 14). Durofume was found extremely effectual against weeds and root-knot nematode i.e. *Meloidogyne incognita*. Considerable decrease in galls formed by nematodes (86.0-96.1%) production was noticed in fumigated plots and egg mass (87.9-97.6%) over control. Likewise, height of plant, leaf yield and leaf moisture were also improved significantly by the soil fumigation and the percentage increase ranged from 3.6 to 6.3, 10.4 to 12.0 and 15.0 to 17.8 respectively. In addition, different weed's population and their densities were significantly decreased in fumigated plots over control (15).

Various different species of plant parasitic nematodes were identified from these study areas and proved the damages caused by parasitic nematodes associated with mulberry (16). Due to that reduction in production yield of the host trees occurs. Infestation of nematodes to host is an unnoticed and slow process and causes serious damage to plant crops, especially in these study areas as temperature is always favourable for reproduction and growth of nematodes. Various different species of parasitic nematodes related to mulberry had been shown from the mixed parasitic culture of nematodes (17). In Mansehra, the population of parasitic nematodes was found to be high as compared to Balakot while in Oghi, species of saprophytic nematodes were found only. Reasons for the less population of parasitic nematodes or absence of parasitic nematodes can be the low temperature of that area or the implementation of old agricultural methods. The population of saprophytic nematodes was very sparse. Implementation of organic fertilizers in fields and around tree trunks is the old agricultural method. For the extermination of the parasitic nematode population, organic fertilizers were found to be very useful (18, 19).

Mulberry trees treated with Carbofuran in Mansehra and Balakot showed a considerable reduction in the population of parasitic nematodes as compared to the parasitic nematode population of the untreated trees (i.e. controlled trees). After 3 months of implementation of the Carbofuran, showed reduction that occurred very early in Mansehra (20). Additional reduction occurred after 6 months implementation whereas after 12 months soil samples were found to be saprophytic. It showed that Carbofuran (nematicide) entirely exterminated the population of plant parasitic nematodes related to mulberry trees. So carbofuran antagonism to parasitic nematodes was highly effective and had an optimistic effect in the yield production and plant health. Whereas, high parasitic nematode population was shown in the controlled (untreated) trees (21-25).

The poultry manure impact on the parasitic nematodes related to mulberry trees in the bars of histogram graphs showed the reduction in the nematode population to a considerable extent in Mansehra and Balakot as well (19, 26). After 3 months of implementation, collected data of parasitic nematodes had shown the decrease in population of parasitic nematodes as compared to untreated trees. More reduction was observed by the same method after 6 months and 12 months of implementation. Though unlike carbofuran, extermination of parasitic nematodes was not entirely but lessened to a considerable extent as compared to the initial population in Mansehra as well as in Balakot. It has been shown that for the control of parasitic nematodes related to mulberry and other plants, poultry manure may be used in Mansehra and Balakot as well (19).

Since poultry manure is eco-friendly as well as organic in nature, thus recommendation of poultry manure is more than carbofuran (nematicide) that is chemical by nature. Poultry manure has not any adverse effect on the environment in decomposition as compared to carbofuran which is toxic and is unfavourable for the environment (26-29).

ANOVA (Analysis of variance) analysis was done on the collected data of the impact of carbofuran on parasitic nematodes related to mulberry statistically in Mansehra and Balakot. The data were analyzed by ANOVA and showed the value of p (i.e. for Mansehra, the value of P was significant P= 0.0001 and for Abbottabad, P value is p= 0.0001). On the other hand poultry manure was also effectual in declining the population of parasitic nematodes related to mulberry. Consequences of ANOVA had shown the poultry manure impact on parasitic nematode population in Mansehra as well as in Balakot found significant also. The value of p was 0.0001 for Mansehra and p=0.0001 for Balakot.

CONCLUSION

It was concluded that the study results confirmed the presence of mixed culture parasitic nematodes in District Mansehra, both parasitic and saprophytic nematode species were present. It was also concluded that in the studied areas, damages were caused by parasitic nematodes and due to less knowledge they were not detected and noticed by farmers and growers. Severe damage caused to growth and yield production of plant. Therefore identification, awareness and control through treatment of the nematodes are essential to improve the yield, growth and food production of plants. It was concluded that both amendments i.e. organic amendment consisting poultry manure and inorganic amendment comprising cabofuran (nematicide) were effectual in declining the parasitic nematode population. Poultry manure declined the parasitic nematode population to the considerable level whereas carbofuran exterminated the parasitic nematode population of nematodes entirely. However, poultry manure is more appropriate and non-toxic in nature than carbofuran that is toxic for environment

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