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MANAGEMENT STRATEGIES TO RESCUE TRANSPLANTABLE VEGETABLES IN AND AROUND YAMUNA RIVER BELT AGAINST HEAVY METALS CONTAMINATION AND SOIL BORNE HIDDEN ENEMIES.-A MATTER OF GREAT CONCERN TO HUMAN HEALTH

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Abstract

Yamuna river belt in Delhi suffers from industrial pollution due to factory effluents and toxic pesticides being indiscriminately used by the farmers causing produces unfit for human consumption. A systematic survey during 2015-2016 around farmer's field at Yamuna river belt showed yield losses of tomato and brinjal by major soil borne fungi caused by root knot nematodes and wilt/rot both alone and together resulting disease complex with synergistic effect on the common hosts. On close interaction with the growers, it was learnt that over use of toxic chemical pesticides caused detrimental effect on human health. All the hidden maladies are expressed as symptoms by patchy growth of the affected crops referred as hot spots.

To combat the above problem, an indigenous, integrated, pest and pollution management (IIPPM) techno-package containing sustainable components was applied at nursery and field to the transplantable crops *i.e.*, tomato, brinjal. The package contains specific fungal bio-agent against respective pathogens, three species of Brassica namely *B. juncea*, *B. napus* and *B. rapa* against bioremediation, *Eclypta alba* and *Phyllanthus niruri* as bio-fumigant botanicals. The three species of *Brassica* also served as trap crops, trapping the heavy metals from environment. The treatment proved to be highly successful as it decreases the nematode level to less than 2 larvae/gram soil (ETL) and simultaneously reducing the heavy metal of soil to significant level. *Brassica juncea* having proven to be the most effective among three species under investigation and is recommended for crop rotation with other vegetables.

Key words : Industrial pollution, root knot nematodes, hot spots, bioremediation.

Introduction

Green revolution in India has welcomed large scale production of hybrid varieties and focused only on crop production but have neglected crop protection issues. As a result, the uneducated growers in India being unaware are still using a large dose of banned pesticides and fertilizers *i.e.*, Endosulfan. In addition the same field has also become victimized by the ingress of heavy metals coming from industries. The seriousness of the contamination was highlighted in a study undertaken by TERI in 2012 sponsored by UNICEF. It showed how despite government efforts industrial effluents and untreated sewage continue to choke the river. In fact, the toxins have polluted the ground water and soil. It has entered our food chain through the vegetables grown on the belt and continues to affect the people living on the belt. Most of the vegetables being sold at Delhi's wholesale markets are washed in the toxic water of the Yamuna. Others are grown in the bed of trash along it and both kinds pose danger to our health. The vegetables growing in this soil absorb the contaminants. "Vegetables contaminated with such toxins can impact normal health quite seriously. It can cause a range of conditions including cancers, heart disease, brain, kidney and liver diseases, muscle and general weakness," Dr. S.P. Byotra, Head of Internal Medicine at Sir Ganga Ram Hospital said.

Vegetables contaminated with such toxins have a huge negative impact on human health. It can cause a range of conditions including cancer as well as lung, heart, brain, kidney, liver diseases, reproduction failure (partial sterility/ full sterility), muscle and general weaknesses as said by

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Dr. S. P. Byotra, head of internal medicine, Sir Ganga Ram Hospilt also quotes a 2012 study by National Reference Trace Organics Laboratory and Central Pollution Control Board (CPCB) in association with the environment ministry that found Lindane, a carcinogenic insecticide in Yamuna watertal. In 2012, a study by National reference trace organics laboratory and Central pollution control Board in association with environment ministry was done and a carcinogenic insecticide, Lindane was found in Yamuna water.

A systematic survey was conducted on 2015-2016 at the grower's field in the Geeta Colony site of River Belt. Growers were facing yield-losses of Solanaceous crops *viz.*, Tomato, Brinjal, Chilli by soil borne fungi causing wilt, rot along with root knot nematodes.

Soil borne fungi along with RKN are certain hidden enemies in the soil for which growers are suffering huge yield loss. Interaction between fungi and nematodes having been recognized since 1892, when Atkinson reported Fusarium wilt of cotton and it was found to be more severe in presence of root-knot nematodes (*Meloidogyne* sp.) than in their absence. Several workers have been reviewed the work on interaction of plant parasitic nematodes with fungi on various crops (Castillo *et al*., 2003; Goswami *et al*., 2007). Root knot nematode (*Meloidogyne* sp.) is reported by the above mentioned authors to predispose the attack of root wilt (*Fusarium* sp.) and attack of root rot (*Rhizoctonia* sp.) and form disease complex which goes very deep in the soil showing synergistic interaction and reduces the soil quality and yield. Being totally ignorant, the growers keep on increasing the doses of chemical pesticides mainly organophosphates and carbamates which are very toxic with high residual effect creating potential hazard to the environment and finally the health of higher animals including human beings wrecking their immune systems.

Scanning of literature clearly reveals no safe and cost effective solution for the above mentioned maladies including soil borne ones and environmental pollution posing a continuous threat to human health. It is therefore, felt desirable to investigate a safe and cost effective Indigenous integrated pest and pollution management package (IIPPM).

Components of IIPPM

Vermi-compost

Vermi-compost has been found to effectively enhance the root formation, elongation of stem and production of biomass in vegetables, ornamental plants etc. (Grappelli *et al*., 1985; Bano *et al*., 1993; Atiyeh *et al*., 1999). The nutrient level in the vermi-compost, especially the macro- and micronutrients were found to be always higher than the compost derived from other methods. One of the unique features of vermi-compost is that during the process of conversion of various organic wastes by earthworms, many of the nutrients are changed to their available forms in order to make them easily utilizable by plants. Therefore, vermi-compost has higher levels of available nutrients like nitrogen, exchangeable phosphorous and soluble potassium, calcium and magnesium derived from the wastes (Buchanan *et al*., 1988). These distinctive properties can be utilized in considering vermi-compost as a suitable carrier for the bio-formulation and as a significant component of the proposed package.

Neem oil seed cake

Neem (*Azadirachta indica*) is widely used for management of root knot nematodes infecting vegetables, pulses and soil borne fungus causing wilt, root rot etc (Sharma and Bedi, 1988). It has also a strong insecticidal property.

Biopesticide

As potential antagonistic fungal bio-agents, the filamentous Deuteromycetes fungi, *Trichoderma harzianum*, *Paecilomyces lilacinus* (Khan & Goswami, 2002) and *Aspergillus niger* (Zuckermann, 1994) have attracted attention due to their multi prolonged action against various soil borne pathogens including root knot nematodes. The species of *Trichoderma* have been evaluated against wilt, root rot fungi, and root knot nematode under glass house and field conditions and found to give results (Goswami *et al*., 2005). Several isolates of *Paecilomyces* showed antagonism against root knot nematode (Goswami *et al*., 2005) and the application of several isolates of *Trichoderma* and *Paecilomyces* against pathogenic soil borne fungi and root knot nematode showed outstanding results in combating soil borne diseases. Local botanicals *i.e.*, *Phyllanthus niruri* and *Eclypta alba* grown as weeds are also components of the package as they are reported to have nematicidal as well as fungicidal property (Goswami *et al*., 2013).

Besides in the present work, three species of *Brassica* namely *Brassica juncea, Brassica napus* and *Brassica rapa* have been included. These plants are reported to eliminate heavy metals *i.e.*, Cd, Pb, Ni, Zn from soil and environment and known as good metal accumulators having high biomass and fast growing property and have been evaluated as potential phyto-extraction plants (Akhtar *et al*., 2013). These above plants have also been reported to exhibit bio-fumigant property against soil borne fungi and nematodes

Table 1 : Properties of sustainable bio-components of IIPPM Package.

Components	Bio-remedant	Bio-fumigants	Bio-pesticides	
			Fungicides	Nematocides
Azadirachta indica		√	√	√
Trichoderma harzianum			√	√
Paecilomyces lilacinus				√
Aspergillus niger				√
Phyllanthus niruri		√	√	
Eclypta alba		√	√	
Brassica juncea	√	√	√	√
Brassica napus	√	√	√	√
Brassica rapa	√	√	√	√

(Ravichandra, 2014). Apart from being phyto-extractor, the functional groups present in plant biomass *viz*. acetamido, alcoholic, carbonyl, phenolic, amido, amino, sulphydryl groups etc. have affinity for heavy metal ions to form metal complexes or chelates. The mechanism of bio-absorption process includes chemi-sorption, complexation, adsorption on surface, diffusion through pores and ion exchange etc which make it suitable to add as dried botanicals in the package (Sud *et al*., 2008).

The major components of IIPPM package showing multifarious activities are given in table 1.

Materials and Methods

1. Soil tested for evaluating content of heavy metal using Atomic Absorption Spectrophotometer (AAS) Shimadzu AA 7000 (Gasper and Anton, 2005). Soil sample was also tested for evaluating nematode (Mi) population. The intensity of myco-flora is measured through soil dilution technique expressing consistently occurring fungus *R. solani* or *F. oxysporum*. The former being represented by mycelium alone while the latter would express 2 types of spores micro and macro causing wilt fungus.
2. Identification of hot-spots along the Yamuna belt expressed as symptoms by patchy growth of the affected crops where the areas have been covered by hidden maladies, root knot nematodes alone and together with soil borne fungi (wilt) and bacteria (rot) causing disease complex (fig. 1) and also dumped indiscriminantly by toxic chemical pesticides and heavy metals.
3. Planting trap plants *i.e.*, *Brassica juncea, Brassica napus* and *Brassica rapa* surrounding the hotspots.
4. Identification of the pest, pathogens, weeds and preparing bio-components through illustration to growers and making them aware about the deleterious effects of polluted land, water and usage of chemical pesticides for the management of pests and diseases including root knot nematodes and convincing them about the present proposed IIPPM package at selected hot-spots.
5. After illustration of the weeds namely *Phyllanthus niruri* and *Eclypta alba* and the other botanicals namely *Brassica juncea*, *Brassica napus* and *Brassica rapa* growers will be engaged for collection of all the botanicals, shed drying and crushing to prepare powder to be used in package.
6. Selecting potential strains of fungal bio-agents *Trichoderma harzianum* and *Paecilomyces lilacinus* and *Aspergillus niger* from the lab consortium, wide range of commercially available products to combat the soil borne hidden enemies.

Since, these vegetable crops are transplantable, treatment was done at nursery level:

At nursery level

a. Soil treatment: Fungal bio-agents (*Trichoderma harzianum* + *Paecilomyces lilacinus* + *Aspergillus niger* @50g each/kg) was applied two weeks prior to sowing with about 500g of Vermi-compost containing 50g each of botanicals.
b. Two weeks prior to sowing mixture of fungal bio-agents powder (*Trichoderma harzianum*+ *Paecilomyces lilacinus* + *Aspergillus niger* @50g each) of fungal colonized grains and also 500g of Vermi-compost containing 50 g of each of botanicals was added to nursery bed.
c. Seed treatment: Seeds to be sown after treating with 0.001% $HgCl_2$.
d. Bare root dip treatment : Prior to transplantation of 20-30 days old healthy seedlings in a slurry

Fig. 1 : Patches of vegetable crop showing symptoms of hotspots.

Fig. 2 : Disease complex symptoms showing root knot nematodes,wilt and rot.

containing Jaggery @ 50g+fungal bio-agents (@ 50g each/lit.) dipped for 30 minutes followed by transplantation with about 500g of Vermi-compost containing 50g of each of botanicals.

Transplantation of 4 weeks nursery healthy seedlings (Tomato and Brinjal) to the 'hot- spots' on micro-plot (2*2 sqm) keeping 30 cm distance between the seedlings prior to which deep ploughing along with application of a mixture of fungal bio-agents 50g + 10kg Farm Yard Manure + 5kg neem oilseed cake in powdered form for as spot treatment.

Indigenous mass production on starch rich grains

For the indigenous mass production of fungal bio-agents, overnight soaking of starch rich grains (sorghum) in water was followed by draining out and filling about 100-150gm of the same soaked grains in autoclavable polypropylene bags. The bags was sealed with thread followed by sterilization in pressure cooker on LPG burner for about 4-5 whistles. On cooling, around 10-15 pre-mycelium collected from lab consortium was introduced in fresh packets containing grains by transferring near flame of the lamp followed by prompt sealing. Inoculated poly bags was incubated for 10-12 days at ambient

Fig. 4 : Indegenous grain based technology for preparation of fungal bio-control agent.

temperature till profuse growth following which they will be ready to apply in the fields (fig. 4).

Field (2×2 sq.m) trial was carried out by Randomized Block Design (RBD).

The observations in respect of plant growth parameters, disease incidences and quality in respect to pollution as well as yield of the produces were systematically recorded all through the investigation period. Simultaneously, the interactive session with the concerned growers was maintained and their response was taken into account.

Soil samples and trap plants were collected and tested for evaluating content of heavy metal using Atomic Absorption Spectrophotometer (AAS). Soil sample was also tested for evaluating nematode load per gram soil. Collected plants were rinsed thoroughly in water before kept for drying in blotting sheets. Both plant and soil samples were dried in the oven at 60°C for three days. Dried soil was sieved through 0.2 mm size sieves prior to processing for digestion. Before digestion to analyze heavy metals, each sample was dried at 60°C for 48 h. The process of digestion was carried out in closed system. 2 g of plant and 0.5 g of soil was weighed for digestion. The samples were treated with concentrated HNO_3 and HCl (6:1, v/v) (Mohammed *et al.*, 2012). Samples were heated up at 300°C for 45 min by microwave to digest samples and evaporate solvent followed by cooling at room temperature (24 ± 2°C). The final volume was made up to 25 ml by diluting the digested samples with Milli-Q water. Samples were filtered through Whatman No. 42 filter paper (GE Healthcare Life Science). These solutions were then used for metal analysis.

Table 2 : Heavy metal content of hot spot soil before and after treatment (IIPPM package and phyto-remediation by three species of Brassica) analysed by AAS.

Metals	Initial conc. of metals in soil before treat-ment (mg/kg)	Final conc. of metals in soil after treatment (mg/kg)	Decline in metal content (%)
Pb	789.22 ± 6.11	230.12 ± 2.8	70.84%
Ni	588.01 ± 4.8	95.09 ± 2.4	83.84%

Table 3 : Heavy metal content in the harvested *B. juncea* before and after the treatment (Analysed by AAS).

Metals	Metal conc. in (mg/kg) in *B. juncea* harves-ted from green house	Metal conc. in (mg/kg) in *B. juncea* harvested after phyto-remediation treatment
Pb	Nil	230.12 ± 2.8
Ni	Nil	95.09 ± 2.4

Table 4 : Heavy metal content in the harvested *B. rapa* before and after the treatment (Analysed by AAS).

Metals	Metal conc. in (mg/kg) in *B. rapa* harvested from green house	Metal conc. in (mg/kg) in *B. rapa* harvested after phyto-remedia-tion treatment
Pb	Nil	190.12 ± 2.8
Ni	Nil	78.09 ± 2.4

Table 5 : Heavy metal content in the harvested *B. napus* before and after the treatment (Analysed by AAS).

Metals	Metal conc. in (mg/kg) in *B. napus* harvested from green house	Metal conc. in (mg/kg) in *B napus* harvested after phyto-remediation
Pb	Nil	165.12 ± 3.8
Ni	Nil	65.09 ± 3.5

Results and Discussion

The treatments did not show any negative effects on plant growth. There were significant differences in the height and yield of Tomato and Brinjal plants treated with IIPPM package (fig. 5). Pb and Ni were found to be significant in the selected hot spots (table 2). *B. juncea* was found to be maximum accumulator of heavy metal *viz*. Pb and Ni (table 3) among the three species.


Before treatment **After treatment**


Fig. 5 : Brinjal and tomato leaf before and after treatment.

Decline in metal content was found after the treatment. Pb was found to be 70.84% lower and Ni was found to be 83.84% lower in the soil after the treatment. The treatment proved to be successful as it decreases fungal disease occurrence confirmed by substantial reduction of the spore count in the rhizosphere soil and the nematode level to less than 2 larvae/gram soil (ETL).

From the present study, it is amply clear that IIPPM package along with three trap species of *Brassica* reduces the soil borne hidden maladies and thus improvement in plant height and yield were observed. The package along with the trap plants efficiently up took Pb and Ni from polluted soil at Yamuna belt and so it act as good hyper-accumulator. Although, polluted Yamuna is the main source of contaminated vegetables in Delhi, nothing could be done to safe the vegetables of Yamuna belt as yet. The treatment proved to be highly successful as it decreases the nematode level to less than 2 larvae/ gram soil (ETL) and soil borne pathogens and simultaneously reducing the heavy metal of soil to significant level. *Brassica juncea* having proven be the most effective among three species under investigation and is recommended for crop rotation with other vegetables.

References

Akhtar, Mohd Sayeed, Birtaut Chali and Tanweer Azam (2013). “Bioremediation of Arsenic and Lead by Plants and Microbes from Contaminated Soil.” *Research in Plant Sciences*, **1.3** : 68-73

Atiyeh, R. M., S. Subler, C. A. Edwards and J. Metzger (1999). Growth of tomato plants in horticulture potting media amended with vermi-compost. *Pedobiologia*, **43** : 724-728.

Bano, K., R. D. Kale and G. P. Satyavathi (1993). Vermi-compost as fertilizer for ornamental plants. In: Rajagopal, D., Kale, R. D. and Bano, K. (Ed.) *Proc. IV National Symposium Soil, Biology. Ecology*. ISSBE. UAS, Bangalore. pp. 165-168.

Buchanan, M. A., E. Russelli and S. D. Block (1988). Chemical characterization and nitrogen mineralization potentials of vermi-composts derived from differing oraganic wastes, in Earthworms in Environmental and waste Management, (Eds C. A. Edwards and E. F. Neuhau ser), SPB Acad, Publ., The Netherlands, pp. 231-239.

Castillo, P., N. Vovlas, S. Subbotin and A. Troccoli (2003). A new root-knot nematode, *Meloidogyne baetica* n. sp. (Nematoda: Heteroderidae), parasitizing wild olive in Southern Spain. *Phytopathology*, **93** : 1093–102.

Goswami, B. K., Archana Mittal and Satyendra Singh (2005). Management of disease complex caused by root-knot nematode, *Meloidogyne incognita* and root fungus, *Rhizoctonia solani* on balsam, *Impatiens balsamina* by fungal bio-agents. *Pesticide Research Journal*, **17 (1)** : 19-22.

Goswami, B. K., Rajesh Kr. Pandey, Jaideep Goswami and D. D. Tewari (2007). Management of disease complex caused by root knot nematode and root wilt fungus on pigeonpea through soil organically enriched with Vesicular Arbuscular Mycorrhiza, karanj (*Pongamia pinnata*) oilseed cake and farmyard manure. *Journal of Environmental Science and Health Part-B*, **42(8)** : 899-904.

Goswami, B. K. and Neetu Singh (2013). A growers friendly and Economic IPM Strategy to combat the Soil Borne Fungal and Root-Knot Nematode Diseases Infecting Horticultural crops. *International Journal of Economics and Management Engineering*, **3(6)** : 262-267.

Grappelli, A., U. Tomati and E. Galli (1985). Earthworm casting in plant propagation. *Hortic. Sci*., **20(5)** : 874-876.

Khan, M. R. and B. K. Goswami (2002). Management of root-knot nematode infecting tomato through indigenous isolate of *Paecilomyces lilacinus*. *Int. J. Nematol*., **12(1)** : 82-86.

Máthé-Gáspár, G. and A. Anton (2005). Phyto-remediation study: Factors influencing heavy metal uptake of plants. Proceedings of the 8th Hungarian Congress on Plant Physiology and the 6th Hungarian Conference on Photosynthesis. *Acta Biologica Szegediensis*, **49** : 69-70.

Mohammed, S. S., M. A. Batu and M. B. Mohammed (2012). Analysis of Cr in Dump site Soil Samples Using AAS and EDXRF Techniques. *Res. J. ChemSci.*, **2** : 65-68.

Ravichandra, N. G. (2014). *Horticultural nematology*, ISBN 978-81-322-1841-8.

Sharma, J. R. and P. S. Bedi (1988). "Effect of soil amendment with oil seed cakes on wilt of cotton". *J. Res. PAU*., **25** : 414-416.

Sud, D., G. Mahajan and M. P. Kaur (2008). Agricultural waste material as potential adsorbent for sequestering heavy metal ions from aqueous solutions- a review. *Bio-resource Technology*, **99 (13)** : 6017-6027.

Zuckerman, B. M., M. Matheny, N. Acosta (1994). Control of plant-parasitic nematodes by a nematicidal strain of *Aspergillus niger*. *J. Chem. Ecol*., **20** : 33–43.