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ESTIMATION OF LOSSES CAUSED BY ROOT ROTTING FUNGI AND ROOT KNOT NEMATODES INFECTING SOME IMPORTANT CROPS IN LOWER SINDH AND HUB, BALOCHISTAN OF PAKISTAN

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Abstract

Soil-borne plant diseases caused by root-knot nematodes and root rotting fungi are a serious threat to modern agriculture. In Pakistan, no extensive research work has been done on crop losses estimation by these pathogens and pests. During the present study, survey of diseased agricultural fields, at different locations in Lower Sindh and Hub, Balochistan was carried out in different seasons to determine the losses caused by soil-borne root-rotting fungi and root-knot nematodes in some economic crops like chili (*Capsicum annuum* L.), cotton (*Gossypium hirsutum* L.), egg-plant (*Solanum melongena* L.), melon (*Cucumis melo* L.), sunflower (*Helianthus annuus* L.), tomato (*Lycopersicon esculentum* Mill.) and watermelon (*Citrullus lanatus* (Thunb.) Mansf.). The most common root rot pathogens found in all the areas visited were *Macrophomina phaseolina*, *Rhizoctonia solani* and species of *Fusarium* viz., *Fusarium oxysporum* and *F. solani.* Two species of root-knot nematode *Meloidogyne incognita* and *M. javanica* were also found causing root- knot disease. Loss to chili crop was found upto 36-56% when infected with *F. solani* and *R. solani* combined with root knot nematodes. Whereas loss to melon crop was found 30-60% due to *F.solani* in different areas of Thatta. Association of *Fusarium* spp., with root knot nematodes was found causing 50-85% loss to tomato crop. Charcoal rot caused by *M. phaseolina* was found to be an important disease of sunflower and cotton. Variations in losses due to these pathogens depended upon combination of pathogens and soil types.

Key words: Crop losses, Lower Sindh, Balochistan, Root rotting fungi, Root knot nematode.

Introduction

The yield losses caused by plant pathogens, pests and weeds are estimated between 20 to 40% of global agricultural productivity (Savary *et al*., 2012). Among these, plant parasitic nematodes caused losses of US$ 100 billion per year to world agriculture (Kayani *et al*., 2017, Mukhtar *et al*., 2017a,b). The soil-borne plant pathogens infecting plant roots are one of the most important problem facing world agriculture that reduce yield and quality in economic crops (Aslam *et al*., 2017; Katan, 2017). Among the soil-borne root rotting fungi, *Macrophomina phaseolina*, the charcoal rot fungus, is one of the most destructive pathogen of crop plants in tropical and sub-tropical countries and it became more destructive at high temperature and water stress condition (Khan *et al*., 2007; Rayatpanah *et al*., 2012; Ijaz *et al*., 2013). Charcoal rot caused serious losses in soybean in USA, Brazil, China, India, Canada and Italy and in sorghum it caused 70% losses in Somalia (Vibha, 2016).

Fungi belonging to genus *Fusarium* causes diseases like root rot, crown rot and vascular wilts on more than 2000 plant species. The highly prevalent and destructive species is *Fusarium oxysporum* (Sudhamoy *et al*., 2009). Another important and very common species of *Fusarium* is *F. solani* that causes losses in bean up to 84% in United States (Schneider *et al*., 2001). Due to higher abiotic stresses the disease is more devastating in developing countries (Hagerty *et al*., 2015). Similarly, *Rhizoctonia solani* causes significant yield losses to several important food crops globally (Paulitz *et al*., 2006; Sneh *et al*., 1996). *Rhizoctonia solani* has very high competitive saprophytic ability, colonizes dead organic matters and attacks almost all important crop plants, makes it one of the most important pathogen worldwide, attacking plant roots (Tewoldemedhin *et al*., 2006). Extensive research work on losses caused by soil-borne plant pathogens has not been done in Pakistan. The present report, therefore, describes the estimation of losses caused by root rotting fungi and root-knot nematodes in some important crops grown in Lower Sindh and Hub, Balochistan, Pakistan.

Materials and Methods

Survey of disease fields: A comprehensive survey of diseased fields of 7 economically important crops viz. chili (*Capsicum annuum* L.), cotton (*Gossypium hirsutum* L.), egg-plant (*Solanum melongena* L.), melon (*Cucumis melo* L.), sunflower (*Helianthus annuus* L.), tomato (*Lycopersicon esculentum* Mill.) and watermelon (*Citrullus lanatus* (Thunb.) Matsum. & Nakai were carried out in different seasons at different locations of Lower Sindh and Balochistan including Memon Goth (2 fields), Darsano Chano (1 field), Gadap (10 fields) and Malir (2 fields) from Karachi Division; Gharoo (5 fields), Ladhiha (2 fields), Sacroo (4fields) from District Thatta; Hala (1 field), Hyderabad (1 field), Tandojam (4 fields) from Hyderabad Division; District Mirpur Khas (10 fields); Hub (1 field), Sakran (1 field) and Vinder (2 field) from Balochistan to evaluate the losses caused by soil-borne root-infecting fungi and root-knot nematodes. Farmers were interviewed and losses were calculated by comparing the obtained production with estimated production (production in a healthy crop). Roots samples of diseased plants were collected and brought to the laboratory for isolation and identification of the disease causing organisms. At least 5 samples of infected plant were collected from each field. Samples were kept under refrigeration at 4ºC until the isolation of fungi were made within 24 hours.

Determination of crop losses: The percentage of crop losses by soil-borne plant pathogens was determined by the following formula:

$$\% \text{ Losses} = \frac{\text{Obtained yield (from diseased crop)}}{\text{Yield of healthy crop}} \times 100$$

Isolation of fungi from roots: After washing in running tap water, roots were sterilized with 1% bleach, 1cm long root pieces were transferred on PDA containing penicillin (100000 units/l) and streptomycin (0.2 g/l). The Petri plates were incubated for 5 days at 28ºC to substantiate infection and colonization of roots by soil-borne root infecting fungi. Infection and colonization percentages were calculated as follows:

$$\% \text{ Infection} = \frac{\text{No. of plants infected by a fungus}}{\text{Total number of plants}} \times 100$$

$$\% \text{ Colonization} = \frac{\text{No. of root pieces infected by a fungus}}{\text{Total number of root pieces}} \times 100$$

Identification of root knot nematodes: Roots showing galls were cut into small pieces and dissected under a stereo microscope for the identification of root knot nematode. Pear shaped females found inside the roots were picked (ten from each sample) and transferred onto a glass slide. Perennial pattern of each female was cut with the help of a sharp razor under dissecting microscope and species of *Meloidogyne* were identified after comparing with known pattern of root knot nematodes according to Taylor & Sasser (1978).

Root knot index: Infection of roots by knot forming nematode was estimated using 0-5 scale described by Taylor & Sasser (1978).

Statistical analysis: Analysis of variance (ANOVA) was used to analyze the data and significant level was determined according to Gomes & Gomes (1984).

Results

Soil-borne plant pathogens associated with diseased agricultural fields and estimation of crop losses: Out of seven crop plants examined, *F. solani* and *R. solani* were found infecting all 7 test hosts, whereas, *F. oxysporum* was found associated with 5 hosts and *M. phaseolina* was found associated with 6 hosts. Similarly, among the species of root-knot nematodes, *M. javanica* and *M. incognita* were common species associated with respectively 3 and 2 hosts (Table 1).

The pathogenic fungi associated with diseased roots of chili crops were *F. solani* and *R. solani* responsible up to 36% to 56% losses. Diseases were more severe and caused more loss when associated with root knot nematodes (55% to 76%) (Table 2). *F. solani* and *M. phaseolina* were found associated with roots of diseased and wilted plants of watermelon, causing 30 and 35% losses in Vindar and Sacroo. Most of the melon fields were attacked by a root rot pathogen *F. solani*, causing 30% to 60% losses in different areas of Thatta when severely infected. Several diseases have been reported on sunflower, causing huge losses, but during our survey at different locations in Lower Sindh, charcoal rot caused by *M. phaseolina* was found to be a serious limiting factor in sunflower production. The fungus was also found associated with *F. solani* and *R. solani* causing 30% to 40% losses in yield. *M. phaseolina* was also found associated with cotton roots, retarding the growth of plant and occasionally causing death of individual plants. Great losses (50% to 85%) were recorded in tomato caused by *F. oxysporum* and *F. solani* with association of root knot nematodes *M. javanica* and *M. incognita*. In eggplant, losses were much higher up to 20% to 55% in Malir, Darsano Chanoo, Memon Goth and Hub when associated with root knot nematode *M. javanica* (Table 2).

Discussion

Fungi and root-knot nematodes attacking roots of crop plants in Pakistan have been reported (Zarina & Shahina, 2010; Hussain *et al*., 2016; Kayani & Mukhtar, 2018; Mukhtar, 2018). The most common root rot pathogens found in all visited areas were *M. phaseolina*, *R. solani* and two species of *Fusarium* namely *F. oxysporum* and *F. solani*. While two species of root knot nematode *M. incognita* and *M. javanica* were also found causing root knot disease.

F. solani and *R. solani* were found responsible up to 36-40% losses in chili. Where *R. solani* affects seedling establishment and was found to cause yield loss of up to 30% (Kataria & Verma, 1992; Khangura *et al*., 1999). Diseases were more severe and caused greater loss when associated with root knot nematode (55-76%). In melon -, *F. solani* was causing 30-60% losses in different areas of Thatta, when severely infected. Charcoal rot caused by *M. phaseolina* was found to be a serious limiting factor in sunflower production. Jalaluddin *et al*., (2008) reported that *M. phaseolina* caused charcoal rot diseases in sunflower. It can infect more than 500 different hosts (Wyllie, 1993; Khan, 2007). The charcoal rot fungus *M. phaseolina* have been reported to attack the root and basal stem of the sunflower, caused root rot disease which results in premature ripening of fruit leading to small head, poorly filled seeds and much reduced yield (Sackston, 1981; Korejo *et al.*, 2019). Great losses (60-85%) were recorded in tomato caused by *F. oxysporum* and *F. solani* with association of root knot nematode *M. javanica* and *M. incognita*. Diseases were much severe in Malir and Memon Goth areas. Root knot nematode problem in Pakistan is common and caused losses of crop plants because climate condition of Pakistan is very suitable for nematode infection (Khan & Ahmad, 2000). Oerke (2006) estimated crops losses due to pests were about 26%-20% for soybean, wheat and cotton and 31%, 37% and 40% for maize, rice and potato. Similarly, Hussain *et al.* (2012) has been reported serious losses due to root-knot nematode in okra from Punjab, Pakistan.

Table 1. Major root rot pathogens associated with some economic crops in different areas of lower Sindh and Balochistan.

Name of crops	Location	Major pathogens associated with roots	Infection % of pathogens	Colonization % of Fungal pathogens & RKI of root knot nematodes
Chili (*Capsicum annuum*)	Sacroo, Thatta	*Fusarium oxysporum*	40	08
		****F. solani*	100	76
		Macrophomina phaseolina	20	04
		****Rhizoctonia solani*	80	20
		Meloidogyne javanica	20	(RKi-2)
	Gharoo, Thatta	*Fusarium oxysporum*	20	04
		****F. solani*	100	80
		****Rhizoctonia solani*	80	28
	Malir, Karachi	*Fusarium oxysporum*	80	28
		****F. solani*	100	84
		Rhizoctonia solani	20	08
		****Meloidogyne javanica*	80	(RKI-5)
	Ladhia, Thatta	*Fusarium oxysporum*	20	04
		****F. solani*	100	72
		Macrophomina phaseolina	20	08
		****Rhizoctonia solani*	60	20
Watermelon (*Citrullus lanatus*)	Vindar, Baluchistan	****Fusarium solani*	80	28
		****Macrophomina phaseolina*	80	36
	Sacroo, Thatta	****Fusarium solani*	80	36
		Macrophomina phaseolina	20	08
		****Rhizoctonia solani*	80	32
	Gharoo	****Fusarium solani*	80	28
		****Macrophomina phaseolina*	80	20
		Rhizoctonia solani	30	08
Melon (*Cucumis melo*)	Gharoo, Thatta	****Fusarium solani*	100	84
		Rhizoctonia solani	20	08
	Ladhia, Thatta	*Fusarium oxysporum*	20	04
		****F. solani*	100	76
		Rhizoctonia solani	40	12
	Sacroo, Thatta	*Fusarium oxysporum*	40	08
		****F. solani*	100	84
		Rhizoctonia solani	40	20
Cotton (*Gossypium hirsutum*)	Gharoo, Thatta	****Fusariumsolani*	80	40
		****Macrophomina phaseolina*	100	68
		Rhizoctonia solani	40	20
	Tandojam, Hyderabad	****Fusarium solani*	80	40
		****Macrophomina phaseolina*	80	52
		Rhizoctonia solani	20	04
	Vindar, Baluchistan	****Fusarium solani*	60	28
		****Macrophomina phaseolina*	100	52
		Rhizoctonia solani	80	44
Sunflower (*Helianthus annuus*)	Gharoo, Thatta	*Fusarium oxysporum*	40	08
		Fusarium solani	20	08
		****Macrophomina phaseolina*	100	68
		Rhizoctonia solani	20	04
	Hala, Hyderabad	*Fusarium oxysporum*	20	04
		****Fusarium solani*	80	48
		****Macrophomina phaseolina*	100	54
		Rhizoctonia solani	20	04
	Tandojam, Hyderabad	*Fusarium oxysporum*	20	04
		****Fusarium solani*	80	28
		****Macrophomina phaseolina*	80	36
		Rhizoctonia solani	20	08

Table 1. (Cont'd.).

Name of crops	Location	Major pathogens associated with roots	Infection % of pathogens	Colonization % of Fungal pathogens & RKI of root knot nematodes
Tomato (*Lycopersicon esculentum*)	Sacroo, Thatta	*Fusarium moniliforme*	20	04
		****F. oxysporum*	100	48
		****F. solani*	80	36
		Macrophomina phaseolina	20	08
		Rhizoctonia solani	20	04
		Meloidogyne javanica	20	(RKI-3)
	Gharoo, Thatta	*Fusarium moniliforme*	20	08
		****F. oxysporum*	100	64
		****F. solani*	80	36
		Macrophomina phaseolina	20	04
		Rhizoctonia solani	40	12
		Meloidogyne javanica	40	(RKI-3)
	Memon Goth, Karachi	*Fusarium moniliforme*	20	04
		****F. oxysporum*	100	52
		****F. solani*	100	44
		Macrophomina phaseolina	20	04
		Rhizoctonia solani	40	16
		****Meloidogyne incognita*	100	(RKI-5)
		****M. javanica*	100	(RKI-5)
	Malir, Karachi	*Fusarium moniliforme*	20	04
		****F. oxysporum*	100	44
		****F. solani*	100	68
		Macrophomina phaseolina	20	08
		Rhizoctonia solani	40	12
		****Meloidogyne incognita*	100	(RKI-5)
		****M. javanica*	100	(RKI-5)
(Eggplant) (*Solanum melongena*)	Memon Goth, Karachi	****Fusarium oxysporum*	80	28
		****F. solani*	100	52
		Macrophomina phaseolina	20	08
		Rhizoctonia solani	40	12
		Meloidogyne incognita	60	(RKI-4)
		****M. javanica*	100	(RKI-5)
	Malir, Karachi	****Fusarium oxysporum*	100	36
		****F. solani*	100	64
		Macrophomina phaseolina	20	04
		Rhizoctonia solani	40	12
		****Meloidogyne incognita*	80	(RKI-5)
		****M. javanica*	100	(RKI-5)
	DarsanoChanoo, Karachi	*Fusarium oxysporum*	40	12
		****F. solani*	100	60
		Macrophomina phaseolina	20	04
		Rhizoctonia solani	40	16
		****Meloidogyne incognita*	80	(RKI-5)
		****M. javanica*	100	(RKI-5)
	Hub, Balochistan	*Fusarium oxysporum*	20	04
		****F. solani*	100	68
		Macrophomina phaseolina	40	12
		Rhizoctonia solani	80	28
		Meloidogyne incognita	60	(RKI-3)
		****M. javanica*	100	(RKI-4)

*** Impact of pathogens were found highly significant ($p<0.001$) on host plants
RKI= Root Knot Index

Table 2. Losses caused by soil-borne pathogens in some economic crops in different areas of lower Sindh and Balochistan.

	Location	Area of cultivation	Obtained yield (kg)	Standard yield (kg)	Losses %
Chili (*Capsicum annuum*)	Sacroo, Thatta	2 acres	550	1250	56*
	Gharoo, Thatta	12 acres	9000	15000	40*
	Malir, Karachi	3 acres	800	3300	76*
	Ladhiha, Thatta	1 acre	700	1100	36*
Watermelon (*Citrullus lanatus*)	Vindar, Balochistan	12 acres	60,000	85,000	30*
	Sacroo, Thatta	20 acres	90,000	140,000	35*
	Gharoo	18 acres	100,000	120,000	17*
Melon (*Cucumis melo*)	Gharoo, Thatta	20 acres	65,000	108, 000	40*
	Ladhiha	40 acres	150, 000	216,000	30*
	Sacroo, Thatta	30 acres	65,000	162, 000	60*
Cotton (*Gossypium hirsutum*)	Gharoo, Thatta	20 acres	6000	8500	30***
	Tandojam, Hydrabad	6 acres	2000	2500	20***
	Vindar, Balochistan	12 acres	4500	5000	10***
Sunflower (*Helianthus annuus*)	Gharoo, Thatta	20 acres	8500	12000	30*
	Hala, Hydrabad	10 acres	4500	7500	40*
	Tandojam, Hydrabad	15 acres	900	13000	30*
Tomato (*Lycopersicon esculentum*)	Sacroo, Thatta	3 acres	750	1500	50**
	Gharoo, Thatta	6 acres	1200	3000	60**
	Memon Goth, Karachi	2 acres	600	3000	80**
	Malir, Karachi	5 acres	750	5000	85**
Eggplant (*Solanum melongena*)	Memon Goth, Karachi	6 acres	2700	6000	55*
	Malir, Karachi	3 acres	2000	3600	45*
	Darsano Chanoo	3 acres	1500	2500	40*
	Hub	15 acres	10,000	12,500	20*

* Significant at p<0.05
** Significant at p<0.01
*** Significant at p<0.001

The common occurrence of these root rot pathogens in Pakistan is presumably due to a temperature of 25-35ºC favorable for the growth of these pathogens which prevails in most part of the year. A temperature of 28-35ºC is optimum for the growth of *M. phaseolina* (Dhingra & Sinclair, 1978), 26-30ºC for *R. solani* (Papavizas & Klag, 1970), 25-30ºC for *F. oxysporum* and 27-30ºC for *F. solani* (Domsch *et al*., 1980). Whereas, the root knot nematodes (*Meloidogyne* spp.) alone are reported to cause 5% loss on world-wide basis (Cetintas & Yarba, 2010)which is much higher in tropical and subtropical countries (Kiewnick & Sikora, 2006). Severe infestation of fields with nematodes like *Meloidogyne* spp. or *Heterodera glycine* can often result in annual losses of 10-50 % (Mc Sorley, 1987; Sasser & Frackman, 1987). The yield to okra, tomato and brinjal suffered 90.9, 46.2 and 27.3% losses respectively due to *M. incognita* infestation @ 3-4 larvae per g soil under field conditions (Bhatti, 1994). Disease is more serious in areas with warm or hot climates and short or mild winters (Agrios, 2005). The association of plant parasitic nematodes with a fungus is reported to cause greater losses than a pathogen alone (Rivera & Aballay, 2008). In this study mixed infection of root rotting fungi with root knot nematodes was found to cause huge losses in tomato. The Association of *F. oxysporum* and *Meloidogyne* are known to increase disease severity in cotton (Starr *et al*., 1989), in tomato (Mai & Abawi, 1987) and in other crops (Fateh *et al*., 2017). Proper estimation of damages caused by the plant pathogens is necessary for the evaluation of losses in each crop and type of pathogens involved. This information is necessary for the development of control strategies and finance required for this program.

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