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STUDIES ON THE DETECTION OF POTATO CYST NEMATODE (*GLOBODERA* SPP.) AND SOIL HEALTH STATUS IN POTATO CORE AREAS OF PUNJAB, PAKISTAN

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ABSTRACT

Potato (*Solanum tuberosum*) is considered as staple food and ranks fourth after wheat, rice and maize in Pakistan. Many biotic constraints affect potato production as potato cyst nematodes (*Globodera* spp.) are considered to be the most important quarantine pests of potato posing serious potential economic consequences throughout the world. Two hundred soil samples collected from Okara, Sahiwal, Kasur and Pakpattan districts were analyzed by floatation technique at Plant Pathology Research Institute, Faisalabad and were found free from potato cyst nematodes infestation. Soil health study was also carried out following standard analytical techniques and soil health indicators for arable land like organic matter, extractable potash, available phosphorus and microbial populations were found in sufficient levels to ensure healthy crop production.

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INTRODUCTION

Potato has become an important strategic crop of Pakistan as it is grown in many areas including higher altitudes (valleys, trenches, slopes etc.) and irrigated plains. Nature has blessed Punjab province with vast agricultural resources on account of its fertile land, canal irrigation system, extremes of weather and centuries old tradition of farming. In Pakistan, potato production was recorded 3831.7 thousand tons during 2016-17 and Punjab has contributed a major share by producing 3660.3 thousand tons of potato (Anonymous, 2017). About 75% of potatoes are grown in Okara, Sahiwal, Kasur and Pakpattan districts which are considered as core areas for quality potato production. Pakistan is expected to harvest over four million tons of potatoes this season as against 3.8 million tons during the last year (2016-17). After meeting the domestic demand of around 2.5 million tons, the marketable surplus averages over 1.5 million tons which will be available for export with a potential to generate revenue of around Rs. 20 billion. Russia, Sri Lanka, Malaysia, Central Asia and Middle East are the big markets for Pakistani potato whose quality and shelf life is better than the Indian potato.

There are many biotic constraints like fungi, bacteria, viruses and nematodes that are potential threats to potatoes. Among these pests, potato cyst nematode, PCN, (*Globodera rostochiensis* and *G. pallida*) are considered to be the most important nematode threat to potato

production worldwide. They cause economic losses on Solanaceae crops due to plant chlorosis, stunting, and general poor growth (Luc et al., 1995) while the level of damage caused by PCN can reach 50-75% (Hadisoeganda, 2006). PCN is a soil borne pest that is spread by infested seed and soil. Due to development of cysts which enclose the eggs, the control of PCN is difficult. Cysts also aid in their dispersal in soil from the infested fields. Soils which are suitable for potato production are also considered good for nematode development.

PCNs were probably introduced in Europe from South America around 1850 by soils adhering to seed tubers of potatoes (Hockland et al., 2012). From Europe, PCN spread throughout the world to almost all areas suitable for potato production. It is more likely that PCN have spread with seed potatoes, soil adhering to tubers from infested fields and by any other means that transport soil containing cysts (Turner and Evans, 1998). PCNs are worldwide in distribution and occur in both temperate and tropical countries (at higher altitudes) of Asia, Africa, North America, South America, Central America, Europe and Oceania where their distribution is linked with potato crop. This pattern of distribution means that PCNs are nematodes of the highest biosecurity and quarantine concern; hence their movement is being regulated by over 100 countries (Lehman, 2004).

Keeping in view the economic and quarantine importance of this pest, Department of Agriculture, Government of Punjab took keen interest to carry out investigations on PCN detection and soil health status of potato core areas of the Province. Therefore, the present studies were conducted to determine the status of PCN in the core potato growing areas of Pakistan.

MATERIALS AND METHODS

Sample collection: Two hundred soil samples from potato core areas (Sahiwal, Pakpattan, Kasur and Okara districts) were collected by technical staff of Potato Research Institute, Sahiwal using sampling augers at a depth of 15-30 cm from postharvest potato fields. A composite sample weighing about 1 kg was collected by taking 8-10 subsamples per field in a zigzag sampling design (Barker, 1985). The collected samples were kept in polythene bags and labeled with sampling details. The samples were brought to Plant Nematology Lab, Plant Pathology Research Institute, Faisalabad and were stored at temperature of 4±2ºC in a refrigerator.

Detection of cysts: For PCN detection, 500 g subsamples were taken from each soil sample, air dried at room temperature and processed by floatation technique (Shepherd, 1986). Hundred gram air dried soil along with 250 ml water were added to each of 500 ml conical flasks, stirred for 20 minutes on shaker at 200 rpm and allowed to stand for 15 minutes. The water was cleared and supernatant contained only floating organic debris and cysts if present. The supernatant was poured off into a funnel with filter paper. This process was repeated four times for each soil sample using separate funnel and filter paper each time. The material collected on filter paper was checked under stereomicroscope (4×10×) for the detection of PCN cysts on morphological basis (Golden, 1986).

Soil health analysis: Soil health studies of 200 samples were carried out in Soil and Water Testing Laboratory and Soil Bacteriology Laboratory, Ayub Agricultural Research Institute, Faisalabad. Soil samples were dried and 100 g subsample from each sample was processed for soil health parameters by following standard analytical techniques viz. organic matter, total organic carbon content (Walkley, 1947), (K) extractable potassium (Richards, 1954), (P) available phosphorus (Olsen et al., 1954), soil pH (Schofield and Taylor, 1955) and soil texture (Bouyoucos, 1962). Soil microbes were isolated from samples (preserved in refrigerator) by preparing the serial dilutions (APHA, 2005; Hedges, 2002). Samples of above mentioned locations were checked on different selective media such as Jensen's, N-free biotin, yeast dextrose calcium carbonate and Pikovskaya's and NBRIP media for the growth of *Azotobacter*, *Azospirillum*, *Pseudomonas* and *Bacillus* spp. respectively. Auxin biosynthesis potential of the above mentioned soil samples was determined by following the technique described by Sarwar et al. (1992). The soil was incubated in sterilized GPM for a period of one week. After incubation period, the medium was centrifuged @ 1000 rpm for half an hour. The supernatant so obtained was processed using Salkowski's reagent and absorbance was noted at 535nm (Sarwar et al., 1992).

RESULTS

No cysts of PCN (*G. rostochiensis* or *G. pallida*) were detected from any of the 200 samples from four core districts (Table1). This reflects that PCN is absent in potato core areas of Punjab. It must be recognized that the results shown refer to the samples received in the laboratory for analysis. Absence of PCN in potato core areas might be due to subtropical nature of the region as environmental conditions may not be suitable for biology

and development of *Globodera* spp. Daily temperature of Sahiwal, Okara and Kasur districts were collected from their respective meteorological stations. However, temperature of Pakpattan is not available due to lack of cited station. As Pakpattan is quite adjacent to Sahiwal, therefore, temperatures are considered to be much similar to recorded in Sahiwal. According to meteorological data, average higher temperature recorded in the month of October for the year 2014, 2015, 2016 and 2017 were 38.9ºC, 33.7ºC, 35.2ºC and 35ºC respectively in Okara (Figure 1). Similarly, higher temperature range of 33.5ºC to 36.6ºC was recorded in Kasur (Figure 2) and that of 32.6ºC to 38.2ºC in Sahiwal (Figure 3) during the last four years (2014-2017).

Table 1. Analysis of 200 soil samples for the detection potato cyst nematode (*Globodera* spp), microbial and hormonal presence in soils of potato core areas of Punjab.

S. No	District	No of cysts/100g soil *(Globodera spp)*	CFU @ 10^7 g^{-1} soil	IAA Equivalents in soil (µg mL^{-1})
1	Sahiwal	0	69.5	1.90
2	Pakpattan	0	62.8	1.69
3	Okara	0	57.6	1.30
4	Kasur	0	47.6	1.22

Data are means of 50 samples for each parameter

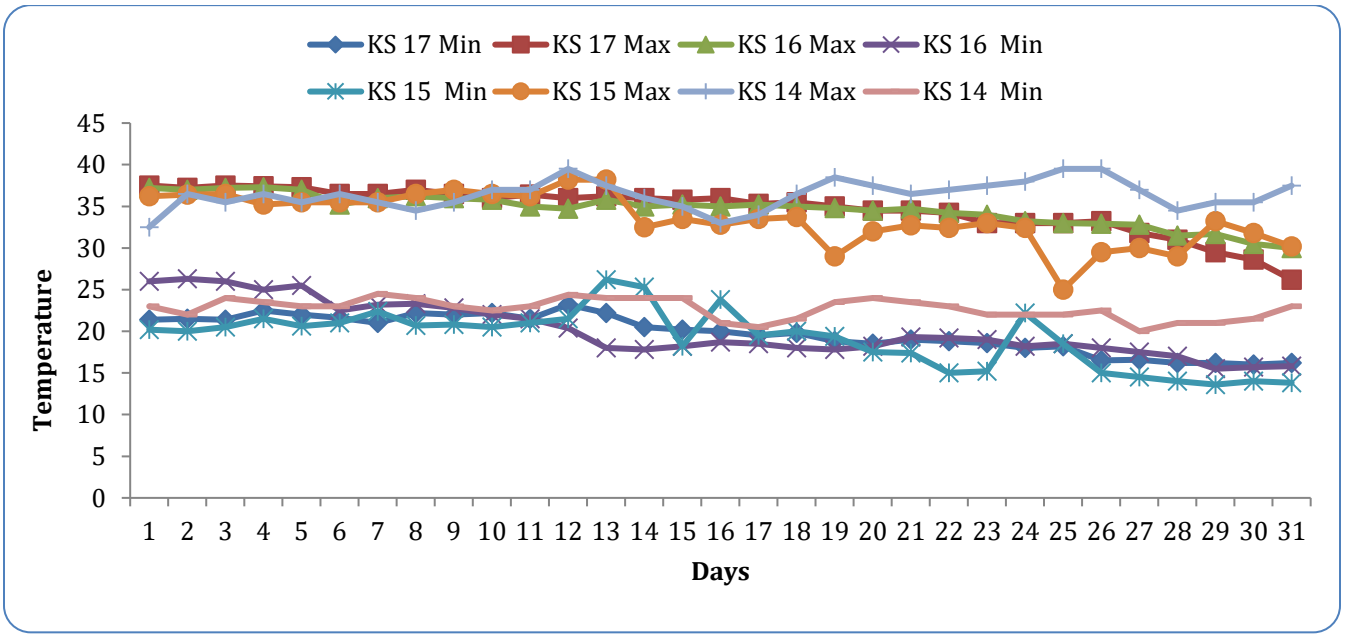

Figure 1: Maximum and minimum temperature recorded in Kasur for the month October in 2014, 2015, 2016 and 2017.

Table 2. Determination of soil health parameters like organic matter, available potash and phosphorus among 200 soil samples collected from four major potato growing districts of Punjab.

`	Okara		Sahiwal		Pakpattan		Kasur	
	Range	Average	Range	Average	Range	Average	Range	Average
Organic matter	0.49-1.40	0.96	0.49-1.40	0.79	0.56-1.32	1.13	0.49-1.40	1.10
pH	7.9-8.5	8.17	7.8-8.7	8.08	7.9-8.5	8.2	7.9-8.6	8.40
Available P (ppm)	5.7-10.3	7.29	5.2-8.0	6.82	5.6-10.6	7.89	5.9-8.9	7.16
Available K (ppm)	60-380	164	60-380	147.5	80-360	257.5	60-380	170

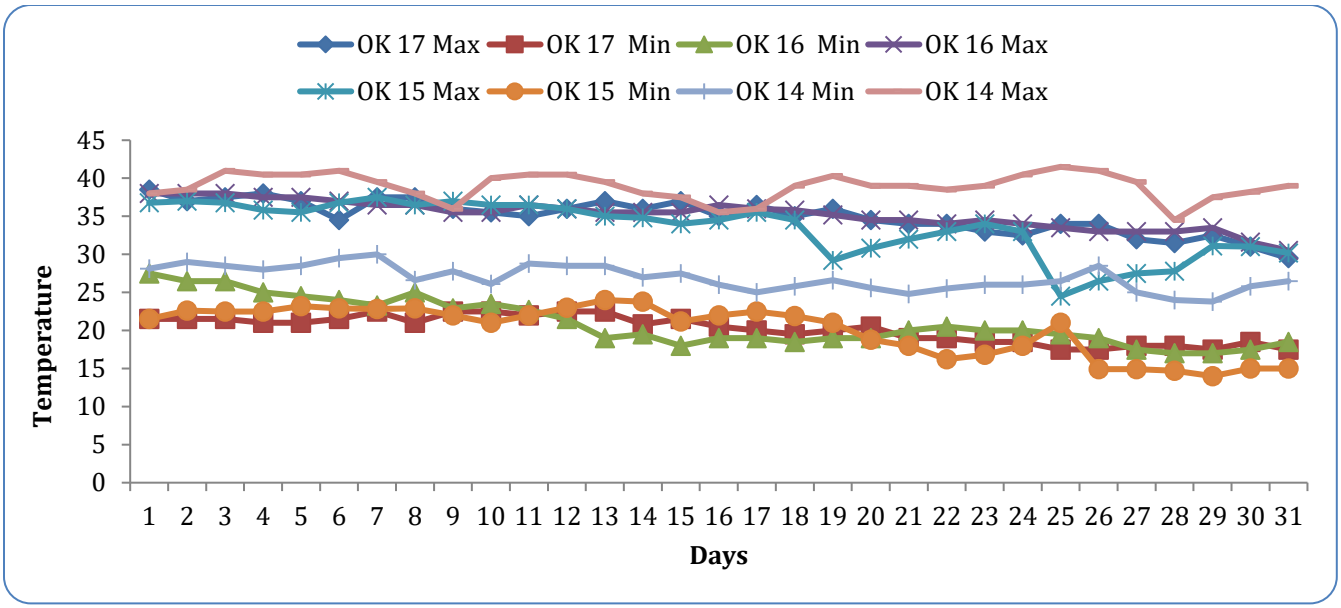

Figure 2. Maximum and minimum temperature recorded in Okara for the month October in 2014, 2015, 2016 and 2017.

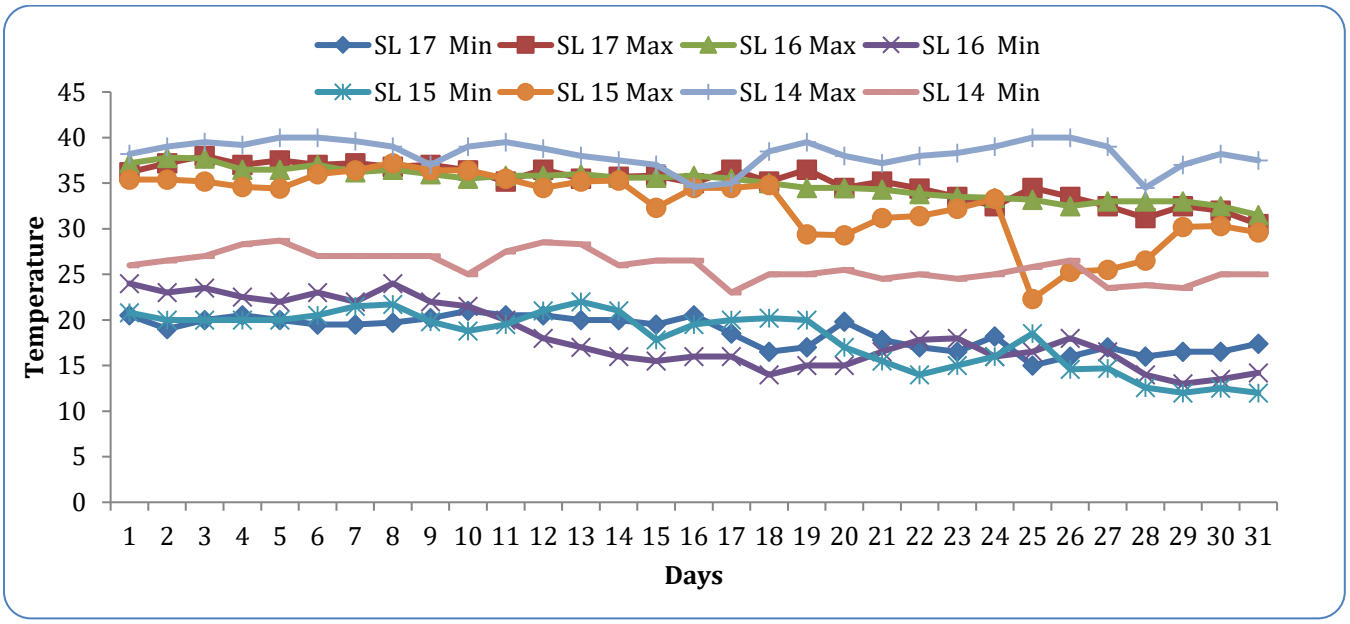

Figure 3. Maximum and minimum temperature recorded in Sahiwal for the month October in 2014, 2015, 2016 and 2017.

Organic matter was found in variable range among soils of four districts as depicted in Table 2. Higher average organic matter content was recorded in soils of Pakpattan (1.13%) followed by Kasur (1.10%), Okara (0.96%) and Sahiwal (0.79%). Soil pH was found alkaline as pH levels were recorded above 8.0 among the tested soil samples. Average potash level was found higher in soils of Pakpattan (257.5 ppm) while lower in Sahiwal (147.5ppm). Similarly, lower levels of available phosphorus were recorded among soils of four districts. Soil texture of the tested samples was found as loamy in nature which is considered good for potato production. Microbial populations in terms of colony forming units/g of soil were found above standard level i.e @ 10^7 cfu/g of soil and recorded in higher levels (69.5×10^7) among Sahiwal soils while lesser numbers (47.6×10^7) were observed in Kasur soils respectively (Table 1). Moreover, Indol Acetic Acid (IAA) equivalents (ug/ ml) in soils of four districts were also determined and found higher in Sahiwal soils (1.905) while lower in Kasur soils (1.22).

DISCUSSION

Presence of PCN was not confirmed in soils of four districts belonging to potato core areas of Punjab province. There may be few reasons for the absence of this pest from this region. Firstly, this pest might have not been introduced into potato core areas of Punjab. Secondly, environmental conditions of potato core areas are not suitable for the development of this nematode. According to several reports, one of the most influential environmental factors affecting nematodes development is temperature (Bacic et al., 2011; Kaczmarek et al., 2014; Van der Waals et al., 2013). According to meteorological data, average higher temperatures recorded for the month of October over a period of four years (2014-2017) in Sahiwal, Kasur and Okara were 35.2ºC, 34.7ºC and 35.6ºC respectively. It is clear from the data, that average higher temperature during the potato sowing period was around 35ºC in potato core areas of Punjab. Hatching of juveniles from cysts is stimulated by potato root exudates released into soil after one week of tubers sowing. Temperature reported above is not favorable for nematode biology and development during potato growing period. Importantly the highest peaks recorded in these potato growing districts during the month of October were ranged as 32.6ºC to 38.9ºC which is not suitable for the development of PCN as reported by other workers. Kaczmarek et al. (2014) conducted a series of experiments on temperature range suitable for hatching and concluded that the greatest cumulative percentage hatch of second stage juveniles (J2s) from cysts occurred between 15-27°C for *G. rostochiensis* and 13-25°C for *G. pallida* while host invasion was inhibited at 30°C. An adverse effect of high soil temperature (above 25ºC) on PCN biology and development was also reported in Belgrade (Bacic et al., 2011). Similarly, annual reduction in nematode population can reach up to 95% where soil temperature was registered above 30ºC (Turner and Evans, 1998). Mortality of nematodes might be greatest under cultivation as higher temperature in arable land were recorded in potato core areas.

Moreover, cropping pattern practiced in potato core areas is not favorable for the multiplication of this nematode as PCN has been reported as exclusive pest on members of Solanaceae family. Generally, Potato - maize - maize - potato, rotation is commonly practiced in this region which is not favorable for PCN development. However, very few farmers practice potato-maize-potato or potato-rice-potato rotations. Therefore, due to non-availability of host crop (Solanaceae family) throughout the year in core areas, multiplication of this nematode is controlled naturally even if present in well below detection level.

In Pakistan, potato cyst nematode (*G. rostochiensis*) was reported for the first time from Khyber Pakhtunkhwa (Maqbool, 1980). Since then this pest has been reported from Northern areas and different parts of Khyber Pakhtunkhwa province by various workers (Gul and Saifullah, 1990; Khan et al., 1987; Khan et al., 1986; Maqbool, 1981; Maqbool and Shahina, 1989; Munir et al., 1994; Shahina and Erum, 2007; Shuja, 1988; Soomro et al., 1995). However, Soomro et al. (1995) could not verify the presence of PCN in Abbotabad and Batakundi as reported earlier by Maqbool (1981) and Gul and Saifullah (1990).

There is only one report about the detection of PCN from Punjab province in Attock, Rawalpindi and Okara areas (Anwar, 1991). Importantly this report could not be confirmed by other researchers as this could just be a misleading report for some reasons. Firstly, the climate in said areas is not suitable for PCN especially temperature may not be favorable for this nematode development. Secondly, the author may have mistaken endo-mycorrhyzal spores or seeds of some weeds as PCN females/cysts as some endo-mycorhizal spores have a shape and color similar to PCN females/cysts. These similarities in gross morphology of spores and cysts have also been observed by various workers and the authors of the present study. These findings are in agreement with those of other workers (Ahmad et al., 1995; Munir et al., 1994; Shahina and Erum, 2007; Soomro et al., 1995) who also could not confirm the presence of PCN in potato core areas of Punjab.

Moreover, presence of PCN in potato core areas of Punjab could also not be confirmed among the tested 77 soil samples received from Federal Plant Protection Department from Okara, Sahiwal and Pakpattan districts in 2016 (unpublished). Analyses of soil samples were carried out in two batches, at Plant Pathology Research Institute, Faisalabad and National Nematological Research Center, Karachi. Analyses reports of said samples were submitted independently by both institutes to Federal Plant Protection Department, Government of Pakistan for quarantine purpose. Importantly, endomycorrhyzal spores confusing with cyst like structures were too observed in abundance by technical staff of both institutes in some soil samples especially

belonging to Pakpattan district.

The presence of adequate levels of organic matter, phosphorus and potash in soils are considered vital for healthy crop production (Ryan et al., 2001). Average organic matter present in soils of Okara, Pakpattan and Kasur districts was found at marginal level (0.86-1.29) but in Sahiwal soils it was recorded at lower level (≤ 0.86). Soil extractable potash was found sufficient as higher levels of potash than adequate level (≥150 ppm) were recorded in soils of four districts. However, soils of potato core areas were found deficient in phosphorus as available phosphorus was recorded in lower level (≤ 8 ppm). Soil texture of the tested samples was found as loamy in nature which is considered good for potato production. Soil pH affects the solubility of ions in the soil and thereby availability of Phosphorus. At high soil pH, increased concentration of calcium results in increased phosphorus fixation. Therefore, high soil pH affects crop growth mainly by reducing the availability of phosphate.

Soil health indicator for arable land is the presence of microbial populations (numbers of colony forming units per gram of soil) and not related to other factors like pH and EC. Levels of microbes reported in analysis depict that soils of Sahiwal district were at top among four districts having sufficient soil microbial levels ideal for better production of crops. Presence of *Azotobacter, Bacillus* and *Pseudomonas* spp. indicates that soils of potato core areas have natural biocontrol agents ideal for healthy production of potatoes. Antagonistic effects of these microbes against soil borne pathogens particularly nematodes is well documented (Beneduzi et al., 2012; Sivasakthi et al., 2014).

Future studies: Potato Cyst Nematodes have the ability to settle in one area and are nematodes of the highest biosecurity and quarantine concern in the world. In the last 100 years, numerous countries have unsuccessfully tried to prevent the introduction of PCN. Even countries with strict restrictions, and those that have attempted eradication, have confirmed infestations of PCN in areas previously free from this nematode viz. USA (Hafez and Sundararaj, 2007), Canada (Sun et al., 2007), Australia (Marshall, 1998). Remote deployment of PCN might be happened through planting of potato seeds, moving of plants and machinery and usage of infected soil and water (Ibrahim et al., 2003; Wang et al., 2001). New incursions of potato cyst nematode have also been reported from Indonesia (Indarti et al., 2004; Nugrahana et al., 2017) and Egypt (Ibrahim et al., 2017) in potato areas located at higher altitudes. These incursions of PCN in new areas indicate that pest could be established in temperate areas of Asian and African countries.

PCNs are soil dwelling pathogens and when sampling a field for nematode detection, a limited amount of soil can be processed. It may be pointed out that in random sampling of wide areas, it is quite possible to miss out the potato cyst nematode particularly if populations are low as their build up is slow. New infestations may therefore, take between 25 and 35 years to develop to a detectable level (Trudgill et al., 2003). Thus it is likely that by the time a new infestation is detected, it might have been spread to other fields through different means. Once established, they are difficult to eradicate because PCNs have one of the highest survival values for any organism and they can survive for over 30 years as eggs are protected by durable cyst wall (Winslow and Wills, 1972). The importance of PCN as pest on potato needs to be cautioned, especially on the potential to be spread into un-infested areas. Therefore, regular surveillance and monitoring to track movement of PCN in potato growing fields must be carried out in order to keep this pest away from potato core areas of Punjab.

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