Prevalence, occurrence and characterisation of disease complex involving *Ralstonia solanacearum* and *Meloidogyne* spp. on potato (*Solanum tuberosum* L.) in eastern Ethiopia

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Accepted for publication 4 May 2022

Summary. A survey was conducted to assess the prevalence, incidence and occurrence of *Meloidogyne* spp. and *Ralstonia solanacearum* (RS) disease complex on potatoes. A total 92 tubers and 92 soil samples were collected from 92 fields. Two species of root-knot nematodes, *Meloidogyne incognita* (MI) and *M. javanica* (MJ), and a bacterial isolate of RS were identified alone or in mixed populations. The highest prevalence of the *Meloidogyne* spp. and RS disease complex was 87% at Kersa. The highest incidence of the disease complex was 70% at Kersa and Chiro. In the tuber samples, the occurrence of the MI, MJ and RS disease complex achieved the highest mean value of 84.6% at Kersa. Farmers' awareness about these pathogens seems to be good. The study confirmed co-occurrence of the *Meloidogyne* spp. and RS in a single host and it indicates the need to examine the importance of these pathogens.

Key words: bioassay, co-occurrence, incidence, *Meloidogyne javanica*, *Meloidogyne incognita*, root-knot nematode, survey.

Potato (*Solanum tuberosum* L.) is the world's third most important food crop after wheat and rice in terms of human consumption (FAOSTAT, 2019). It yields more food more rapidly than any other major crop, provides more nutrition than many other crops, and provides income for many small-scale farmers (Devaux *et al.*, 2020). Unfortunately, the crop suffers from attacks by plant-parasitic nematodes, bacteria and other pathogens, which cause huge losses in production.

Root-knot nematodes (*Meloidogyne* species; RKN) are sedentary endoparasites that are highly polyphagous, global in distribution (Moens *et al.*, 2009), and can cause up to 100% yield losses on tomato (Seid *et al.*, 2015). Overall, plant-parasitic nematodes alone cause an estimated annual crop loss of $US 78 billion worldwide and an average crop yield loss of 10-15%. Nematodes alone can cause average yield losses in potato of up to 12% (Lima *et al.*, 2018).

Prevalance is the proportion of individuals in a population that have a disease at a particular time, regardless of when they were infected, sometimes also called the point prevalence. It is a measure of the disease burden (Campbell & Madden, 1990). Incidence measures the number of new cases of a disease in a population during a given period of time. Occurrence is an instance of disease happening (Madden & Hughes, 1999). In Ethiopia, the prevalence of *Meloidogyne* spp. alone or in mixed populations has been reported on a small number of horticultural crops, such as tomato (Tefera & Hulluka, 2000; Mandefro & Mekete, 2002; Kassie, 2019; Miheret *et al.*, 2019; Seid *et al.*, 2019), hot pepper (Abegaz *et al.*, 2019) and rose (Meressa *et al.*, 2014). Seid *et al.* (2019) reported the prevalence and occurrence of four major *Meloidogyne* spp., *Meloidogyne incognita*, *M. javanica*, *M. arenaria* and *M. hapla* on tomato, using DNA and protein-based identification

 doi: 10.24412/0869-6918-2023-1-17-28
Published online 3 March, 2023

techniques. The occurrence of *M. incognita* and *M. javanica* has also been reported in tomato cultivation areas in eastern Ethiopia (Seid *et al.*, 2019). However, there is limited research information on the incidence, which refers to the rate at which the disease is manifested in a certain population (Hussain *et al.*, 2012), prevalence and occurrence of *Meloidogyne* spp. in major potato growing areas of Ethiopia.

Ralstonia solanacearum (bacterial wilt) is one of the most destructive potato diseases (EPPO, 2020). It is reported as a serious production constraint in Ethiopia, Uganda, Kenya, Madagascar, Rwanda, Burundi, Nigeria and Cameroon (EPPO, 2014). Bacterial wilt in potato has been detected in many parts of Ethiopia (Stewart, 1956; Kassa, 1996; Gebremedhin *et al.*, 2006; Henok *et al.*, 2007; Abdurahman *et al.*, 2017; Tessema *et al.*, 2020). Yield losses of 30 to 100% due to *R. solanacearum* were reported in Kenya and Uganda (Kinyua *et al.*, 2005).

Formation of galls on the root system, suppression of yields as well as overall stunting of the plant, are typical symptoms of *Meloidogyne* spp. (Moens *et al.*, 2009). Wilting and yellowing of the leaves, discoloured tuber eyes, a milky-white sticky exudate or ooze are major symptoms of *R. solanacearum* (Agrios, 2005). The disease complex (synergistic interaction) between *Meloidogyne* spp. and *R. solanacearum* on various hosts is widely recognised (Martin & Nydegger, 1982; Ateka *et al.*, 2001; Pavithra *et al.*, 2014; Sundaresh *et al.*, 2017; Getu *et al.*, 2021). Experiments conducted in glasshouse conditions on various hosts revealed that the damage caused by concomitant infection by *Meloidogyne* spp. and *R. solanacearum* is more severe than that from separate infections (Sundaresh *et al.*, 2017; Getu *et al.*, 2021).

In eastern Ethiopia, research information on the prevalence, incidence and occurrence of *Meloidogyne* spp. and *R. solanacearum*, either alone or as a disease complex on potato, is limited. However, both *Meloidogyne* spp. and *R. solanacearum* are important and increasing constraints to potato cultivation in the region. Therefore, co-occurrence of *Meloidogyne* spp. and *R. solanacearum* may be one of the most important factors responsible for potato yield reduction. The main objective of the present research was to study the prevalence, incidence and occurrence of *Meloidogyne* spp. and *R. solanacearum* disease complex on potato; and to assess the farmers' perception and management practices against these diseases in the main potato growing districts of eastern Ethiopia.

MATERIAL AND METHODS

Description of the study areas. Six districts namely, Haramaya, Kersa, Meta, Kombolcha and Jarso from the east Hararghe Zone and Chiro from west Hararghe Zone of eastern Ethiopia, were included in this survey. The survey was conducted between the 2nd and 4th week of September 2018. In addition, Haramaya University Raree Research Station (HURRS) were included in the survey.

Root and soil sample collection. Tuber and soil samples were collected from the selected potato growing districts of eastern Ethiopia. Potato growing districts were selected after discussion with regional agricultural development agents on the potential of the areas for producing potato. From each district, three potato-growing villages and five farms in each village were surveyed. A total of 184 composite samples from 90 farms and two research stations (92 soil and 92 root or tuber samples) were collected.

Tuber and soil samples were collected aseptically in a zigzag 'Z' sampling pattern, which considers pathogens distribution and nature of the field (Coyne *et al.*, 2018). Samples were from 25 plants per farm, separately so that each tuber sample corresponded with a soil sample. Approximately 1.5 kg tubers per sample were collected. For soil samples, 30 soil core samples were taken from the top 25 cm depth using a 2 cm diam. auger next to the associated plant to make a bulk of about 2 kg soil (Hussey & Barker, 1973). The collected samples were bagged separately in polyethylene bags, tied with a twin thread, and then labelled with necessary field information, and placed immediately inside the ice-box at approximately 5°C for preservation (Coyne *et al.*, 2018). Potato fields about 3 km apart from each other were selected with the help of the regional development agents. During sample collection, all possible care was taken to avoid cross-contamination. The collected samples were transported to Haramaya University and stored in a refrigerator at +5°C to keep the samples fresh (Coyne *et al.*, 2018). The laboratory work was performed in the Postgraduate Plant Pathology Laboratory of Haramaya University, Ethiopia, but the molecular identification of the *Meloidogyne* spp. was carried out at the Nematode Systematics and Biological Control, University of Florida, USA.

For the field survey data collection purpose, semi-structured questionnaires were prepared and provided to all potato growers from whom the samples were collected to assess the farmers' management practices and awareness of *Meloidogyne* spp. and *R. solanacearum* diseases on

potato. Selected interviews and focus group discussions were also made to extract more information about the farmers' practices and awareness of these diseases.

Bioassay test. Soil samples collected from each field were tested to detect the occurrence of *Meloidogyne* spp. and *R. solanacearum* disease complex with a bioassay test using the susceptible tomato 'Marmande' as an indicator plant. Three-week-old tomato seedlings or seedlings that reached the four leaf-stage were grown on all collected soil samples in 500 cm^3 size pots under glasshouse conditions. After 8 weeks, the root system from each pot was collected and was diagnosed for *Meloidogyne* spp. and *R. solanacearum* infection separately.

Nematode extraction. Root-knot females were excised from tuber and tomato root galls using a sterilised scalpel, needle and a stereoscopic microscope. Females dislodged from each tuber and root samples were grouped for each sample in a vial containing water for identification.

Identification of *Meloidogyne* spp. Identification of the species of *Meloidogyne* collected from potato fields was done based on perennial patterns morphology (Taylor & Sasser, 1978; Hunt & Handoo, 2009) and using BLAStn analysis of the primer MORF (ATC GGG GTT TAA TAA TGG G) / MTHIS (AAA TTC AAT TGA AAT TAA TAG C) sequences (Pagan *et al.*, 2015).

***Ralstonia solanacearum* isolation and purification.** *Ralstonia solanacearum* isolation was done from diseased potato tubers showing symptom milky white bacterial cell ooze (Shew & Lucas, 1991) and without clear symptoms, and from the roots of tomato (bioassay test). The samples were macerated, washed with tap water, surface-sterilised in 70% ethanol for 3 min, rinsed in sterilised distilled water and then dried with tissue paper (EPPO, 1990). About 5 g lesions of tuber or root of tomato from each sample were cut aseptically and diseased tissue placed in sterile distilled water, separately. The samples were left to stand for 10 min to allow any bacteria present to be released into a sterile Petri dish containing sterile distilled water; 0.1 ml of the macerate was dispersed on the surface of nutrient agar (NA medium; beef extract 3.0 g, agar 15.0 g, peptone 5.0 g l^{-1}) with a sterile loop and incubated at 28°C for 48 h (Goszczynska *et al.*, 2000).

Presumptive *R. solanacearum* colonies were purified by streaking a single colony of each isolate on 2, 3 and 5-triphenyltetrazolium chloride (TTC solid medium; peptone 10.0 g, casein hydrolysis 1.0 g, glycerol 5 ml, and agar 15.0 g l^{-1}). All the media were sterilised by autoclaving at 121°C for 15 min. In TTC medium, 5 ml of 1%, 2, 3, 5 TTC was added to the sterilised 1 l medium before pouring. It was incubated at 28°C for 48 h (Kelman, 1954).

***Ralstonia solanacearum* identification and culture maintenance.** To identify the bacterial species, the isolates were subjected to morphological, physiological and biochemical tests. Morphological identification was done according to Kelman (1954).

Gram staining. For the Gram staining test, a young 48 h-old bacterial colony was tested to identify whether the isolates were Gram-positive or Gram-negative bacteria. All specimens were examined under an oil immersion microscope (Gregerson, 1978).

KOH solubility test. To see if a mucoid thread can be lifted with the loop or not, a drop, approximately 0.25 ml, of potassium hydroxide (KOH) (3% aq., w/v) was placed using a Pasteur pipette on a clean glass slide (Ryu, 1940). Bacterial cells were transferred from a young culture media aseptically with a loop and placed into the drop of KOH with rapid, circular agitation. After 5-8 sec, the loop was alternatively raised and lowered just off the slide surface to detect a stringing effect. The KOH test was considered positive if drop viscosity or mucoid increased and stringing occurred within 15 s (Suslow *et al.*, 1982).

Oxidative use of carbohydrates. Two test tubes containing basal mineral medium plus 5 ml 10% sucrose solution (w/v) were stab-inoculated with bacteria taken from a young colony. Two drops of sterile mineral oil were added to one tube then the tubes were incubated for 3 weeks to see oxidative reaction (Goszczynska *et al.*, 2000).

Levan production. Levan is a homopolysaccharide of fructose units that is produced by some plants and microorganisms (Gonzalez-Garcinuno *et al.*, 2017). Test tubes containing NA with 5% sucrose were streak-inoculated with young colonies and incubated at 28°C for 5 days. Levan (convex, white, domed and mucoid colonies) production was tested (Schaad, 1980). It was evaluated through observing convex, white, domed and mucoid colonies (Goszczynska *et al.*, 2000).

Decomposition of nitrogenous compounds or arginine dihydrolase. Test tubes containing arginine medium were stab-inoculated with a young bacterial colony then covered with sterile mineral oil and incubated at 28°C. After 48 h the tubes were checked to determine if changes from yellow to pink colour occurred (Goszczynska *et al.*, 2000).

Starch hydrolysis. Starch plates were streak-inoculated with a young 24 h-old bacterial colony,

which was the same for all tests per sample, and incubated for 1 week then flooded with iodine solution (Goszczynska *et al.*, 2000).

Catalase. A few drops of 3% H_2O_2 were added to a 24 h-old bacterial colony on a plate to test for gas bubble formation (Goszczynska *et al.*, 2000).

Biovar tests. To the olive-green coloured basal medium, 10% solution of hexose alcohols (inositol, mannitol, sorbitol) and carbohydrates (cellobiose, sucrose, maltose) were added in 5 ml amounts into sterilised test tubes (150 mm × 10 mm) in 20 ml amounts to group all bacterial isolates into different biovars based on the colour changes. From an already prepared 2-day-old bacterial suspension culture, 0.1 ml was added to two test tubes (control with no carbohydrate). The tubes were incubated at 30°C then examined at 3, 7 and 14 days (Hayward, 1964).

Farmers' awareness and practices towards *Meloidogyne* spp. and *Ralstonia solanacearum*. A cross-sectional analysis study was conducted to assess farmers' awareness and management practices towards *Meloidogyne* spp. and *R. solanacearum*. In the study, a total of 180 respondents (through questionnaire and interview) were incorporated. The independent variables used in the survey were previous crop, irrigation practice, management practice, pesticide used, fertiliser used, source of seed, and level of awareness that potato growers have about *Meloidogyne* spp. and *R. solanacearum* diseases and/ or disease complex. The dependent variables used in the survey were to quantifty *Meloidogyne* spp. and *R. solanacearum* damage symptoms by showing the photos of damaged plants.

Data analysis. The prevalence of *Meloidogyne* spp. and *R. solanacearum* disease complex were assessed after identification at the laboratory by the formula as a total number of fields with disease complex / total number of fields surveyed × 100, while the incidence was determined by the total number of infected plants with disease complex / total number of observed plants × 100 (Hussain *et al.*, 2012). The occurrences of species were computed by number of samples with species / total number of samples observed × 100 (Norton, 1978). The data obtained from the survey and glasshouse (bioassay) were subjected to analysis of variance using SAS software version 9.2 (SAS Institute, 2009) and data of a cross-sectional study were analysed using SPSS version 20 software (SPSS, 2020). The binary logistic regression analysis (odds ratio with corresponding 95% confidence interval) was performed to assess the presence and degree of association between the dependent and independent variables. To observe the significant differences, the mean value of the data was compared using Duncan Multiple Range Test (DMRT) at $P \leq 0.05$ (Gomez & Gomez, 1984).

RESULTS

Identification of *Meloidogyne* spp. The identification of root-knot nematodes using morphological characters of perineal patterns of females extracted from tubers collected from surveyed areas and bioassay tests demonstrated the presence of *M. incognita* and *M. javanica*. The important distinguishing features of perineal patterns of *M. incognita* were typically high with a square-like dorsal arch. Striae were distinct and wavy. The dorsal striae were smooth, closely placed wavy to zigzag; the dorsal and ventral striae appearing to be interrupted and forked at the lateral line. *Meloidogyne javanica* perineal patterns had a low and rounded dorsal arch, lateral ridges that divide the pattern into dorsal and ventral regions or striae. Striae were coarse and smooth to slightly wavy and tail terminus often with distinct curl. *Meloidogyne javanica* was different from other *Meloidogyne* spp. by containing its pattern on the lateral field (Taylor & Sasser, 1978; Hunt & Handoo, 2009). In addition, BLAStn analysis of the MORF/MTHIS sequences (Pagan *et al.*, 2015) showed the tested isolates were all *M. incognita*. Although only 30 (out of 90) samples were identified molecularly, all results supported the identification of *Meloidogyne* spp. by morphological characteristics.

***Ralstonia solanacearum* identification. Morphological.** All *R. solanacearum* isolates produced cream-coloured bacterial colonies on nutrient agar (NA) media after 24 h incubation at 28°C. All isolates produced drop-shaped white colonies with pink, mucoid, or half-moon shapes on TTC media after 48 h incubation at 28°C as described by Kelman (1954).

Physiological. The result of the Gram staining test indicated that all bacterial isolates were Gram-negative (red) (Gregerson, 1978). A mucoid-like viscous thread was lifted from bacterial colony with the help of a loop, and the solution stuck to the loop when touched. This confirmed that the isolates were Gram-negative bacteria (KOH solubility test) (Suslow *et al.*, 1982). A colour change to yellow was observed only in open (without mineral oil) tubes, which confirmed the oxidation reaction; the isolates were identified as *R. solanaceurum* (oxidative use of carbohydrates) (Goszczynska *et al.*, 2000).

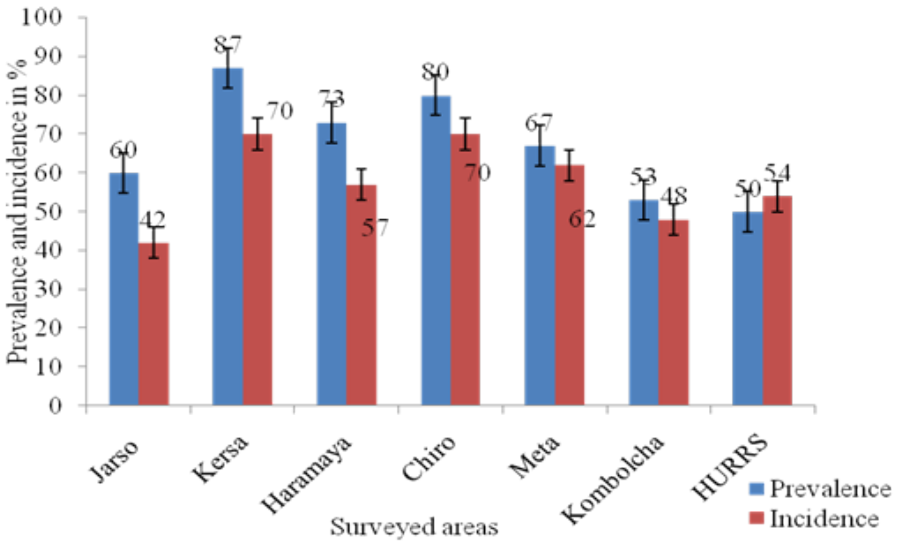

Fig. 1. Prevalence and incidence (%) of *Meloidogyne* spp. and *Ralstonia solanacearum* disease complex in the surveyed areas of eastern Ethiopia. The bar shows standard error at $P \leq 0.05$. HURRS = Haramaya University Raree Research Station.

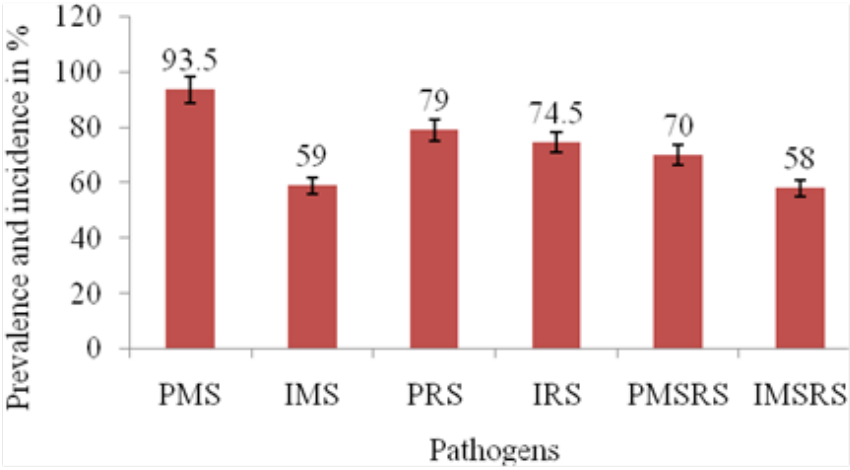

Fig. 2. Overall prevalence and incidence of *Meloidogyne* spp. and *Ralstonia solanacearum* and / or disease complex in the surveyed areas of eastern Ethiopia. PMS = prevalence of *Meloidogyne* spp. (MS), IMS= incidence of MS, PRS = prevalence of *R. solanacearum* (RS), IRS= incidence of RS, PMSRS = prevalence of MS and RS, IMSRS = incidence of MS and RS. The bar shows standard error at $P \leq 0.05$.

Biochemical. Levan production test has been shown that convex, white, domed and mucoid colonies were not produced from sucrose, which shows that the isolates were *R. solanacearum* (Schaad, 1980). After 48 h the tube contents changed from yellow to pink, which confirmed that the bacterial isolates were *R. solanacearum* (decomposition of nitrogenous compounds (arginine dihydrolase)) (Goszczynska *et al.*, 2000). The starch hydrolysis test also showed a blue-black, clear zone around bacterial growth indicating starch hydrolysis (amylase activity) (Goszczynska *et al.*, 2000). The result of the catalase test (formation of a gas bubble) further indicated that the isolates were *R. solanacearum* (Goszczynska *et al.*, 2000). The results with all bacterial isolates indicated that acid pH (yellow colour) was observed from the top downwards of the test tubes between 3 to 5 days. This confirmed the isolates to be biovar II (Hayward, 1964).

Prevalence and incidence of *Meloidogyne* spp. and *Ralstonia solanacearum* disease complex. Out of the 92 tuber and 92 soil samples tested in the bioassay, 78 (85%) and 63 (68.5%) were found to be infested by *Meloidogyne* spp. and *R. solanacearum*, respectively. The prevalence of the *Meloidogyne* spp. and *R. solanacearum* disease complex varied between the surveyed districts with the highest prevalence, 87%, at Kersa as opposed to the 50% at HURRS. In the surveyed areas, the incidence of the disease complex was also variable, with the highest 70% at Kersa and Chiro, and the lowest at Jarso 42% (Fig. 1).

Overall, the prevalence and incidence of the *Meloidogyne* spp. and *R. solanacearum* disease complex in the surveyed areas of eastern Ethiopia were found to be 70% and 58%, respectively. Regardless of disease complex, prevalence of the *Meloidogyne* spp. (93.5%) and *R. solanacearum* (79%) and incidence of the *Meloidogyne* spp. (59%) and *R. solanacearum* (70.4%) were recorded (Fig. 2).

Occurrence of *Meloidogyne* spp. and *Ralstonia solanacearum* disease complex in the tuber and soil samples. *Meloidogyne* spp. and *R. solanacearum* disease complex: *M. incognita* and *R. solanacearum* (MIRS), *M. javanica* and *R. solanacearum* (MJRS), *M. incognita*, *M. javanica* and *R. solanacearum* (MIMJRS) were observed, and varied in the surveyed areas. In the tuber samples, the occurrence of the MIMJRS achieved the highest mean value 34 (84.6%) at Kersa and the lowest 25 (61.6%) at Jarso. The MIRS achieved the highest mean value 29 (72.5%) at Kersa and the lowest of 21 (52.5%) at Jarso. In the soil samples (bioassay test), the occurrence of the MIMJRS achieved the highest mean value 32 (79.1%) at Kersa and the lowest 14 (35.0%) at HURRS. The MIRS achieved the highest mean values 27 (67.5%) at Kersa and the lowest 13 (31.2%) at Jarso. The occurrence of the *Meloidogyne* spp. and *R. solanacearum* disease complex in tubers was found to be higher than in the soil samples (Table 1). Tubers infected by *Meloidogyne* spp. and *R. solanacearum* diseases complex are shown in Figure 3.

Farmers' awareness of and practices against *Meloidogyne* spp. and *Ralstonia solanacearum*. Most of the independent variables, previous crop (Solanaceous), sources of seed (farm saved and research centre), fertiliser (inorganic), farmers' awareness, factors for disease development, and control methods (fungicide, ploughing and crop rotation) had highly significant ($P < 0.01$) effect on the dependent variables. The highest effect on the dependent variable *Meloidogyne* spp. and *R. solanacearum* damage symptoms on potato tuber was from the fertiliser (Table 2). When shown symptoms of *Meloidogyne* spp. and *R. solanacearum*, 163 (90.5%) out of 180 respondents reported damage symptoms such as gall and wilting; the remaing 17 (9.5%) did not report the damage symptoms. Out of the 180 respondents, 133 (74%) and 47 (26%) replied that their field in the previous season was cropped with solanaceous and non-solanaceous crops, respectively. Out of the 180 respondents, 164 (91%), 16 (9%) indicated that in the previous season the solanaceous and non-

Table 1. Altitude range, occurrence of *Meloidogyne incognita*, *M. javanica* and *Ralstonia solanacearum* (MIMJRS), *M. javanica* and *R. solanacearum* (MJRS) and *M. incognita* and *R. solanacearum* (MIRS) disease complex in tuber and soil samples (bioassay test), collected from eastern Ethiopia with total number of fields surveyed per district.

District	Altitude range (m a.s.l.)	Tuber			Soil		
		MIRS (%)	MJRS (%)	MIMJRS (%)	MIRS (%)	MJRS (%)	MIMJRS (%)
Kersa	1942-2075	72.5a(29)	27.5b(11)	84.6a(34)	67.50a(27)	25.8b(10)	79.1a(32)
Haramaya	1991-2115	58.3ab(23)	35.0a(14)	70.0b(28)	54.3bc(22)	32.3a(13)	65.1b(26)
HURRS	2008-2013	65.0b(26)	35.0a(14)	72.5ab(29)	31.2d(13)	18.7c(8)	35.0c(14)
Metta	2013-2131	60.5ab(24)	35.1a(14)	72.1ab(29)	55.3bc(22)	33.6a(13)	66.1b(26)
Chiro	2028-2442	64.3b(26)	35.6a(14)	76.5ab(31)	60.0ab(24)	33.3a(13)	71.3ab(29)
Kombolcha	2084-2197	53.8bc(21)	32.8ab(13)	64.8bc(26)	49.8c(20)	30.1ab(12)	60.0bc(24)
Jarso	2440-2747	52.5bc(21)	27.5b(11)	61.6c(25)	47.6c(19)	25.6b(10)	56.0bc(22)
Mean		60.9(24)	32.64(13)	71.73(29)	52.24(21)	28.49(11)	61.80(25)

Mean values not sharing common letter(s) within columns of each parameter differ significantly at $P \leq 0.05$ according to Duncan Multiple Range Test (DMRT). HURRS = Haramaya University Raree Research Station. Numbers in brackets are mean number of *Meloidogyne* spp. collected and identified from each fields of surveyed districts. m a.s.l. = metres above sea level.

solanaceous crops were grown with irrigation and rainwater, respectively. In those samples collected from where the previous crop was irrigated and in sandy soil type, higher *Meloidogyne* spp. related symptoms were observed (data not presented). The sources of the seeds that respondents used to plant were examined. Out of 180 respondents, seed sources were 103 (57%) farm-saved, 65 (36%) obtained from a research centre, and 12 (6.6%) collected in local markets.

Table 2. Binary logistic regression analysis between the dependant variable (*Meloidogyne* spp. and *Ralstonia solanacearum* damage symptoms) and seven independent variables based on the odds ratio at $P < 0.05$ significance level.

Independent variables	***Meloidogyne* spp. and *Ralstonia solanacearum* damage symptoms**			
	163 (90.5%) 17 (9.5%)	**Odd ratio**	**95% confidence interval**	***P* value**
Previous crop:				
Solanaceous	133 (74%)	0.63	0.51-0.80	< 0.01
Non- solanaceous	47 (26%)	0.87	0.81-0.91	= 0.05
Sources of seed:				
Farm saved	103 (57%)	0.77	0.70-0.88	< 0.01
Research centre	65 (36%)	0.85	0.79-0.92	< 0.01
Local market	12 (6.6%)	0.90	0.85-0.95	> 0.05
Sources of water:				
Irrigation	164 (91%)	164	23-1157	< 0.01
Rain	16 (9%)	0.89	0.85-0.94	> 0.05
Fertilizer:				
Inorganic	165 (92%)	82.5	20.8-327	< 0.01
Organic	15 (8%)	0.89	0.85-0.94	> 0.05
Awareness:				
Meloidogyne spp.	132 (73%)	0.64	0.52-0.79	< 0.01
Ralstonia solanacearum	94 (52%)	0.80	0.72-0.89	< 0.01
Factors to disease development:				
Climate change	135 (75%)	0.62	0.49-0.78	< 0.01
Pathogens	45 (25%)	0.87	0.82-0.93	< 0.01
Controlling methods used:				
Fungicide	141 (86%)	0.59	0.46-0.76	< 0.01
Insecticide	10 (6.4%)	0.90	0.85-0.94	> 0.05
Herbicide	4 (2.5%)	0.90	0.86-0.94	> 0.05
Ploughing	132 (73%)	0.64	0.52-0.79	< 0.01
Crop rotation	117 (65%)	0.73	0.63-0.84	< 0.01
Host plant eradication	22 (12%)	0.89	0.84-0.94	> 0.05
Fallowing	3 (2%)	0.90	0.86-0.94	> 0.05

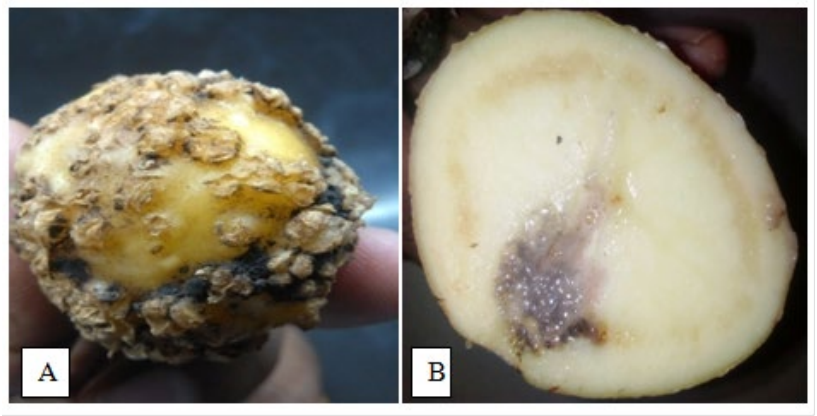

Fig. 3. Tuber infected by *Meloidogyne* spp. and *Ralstonia solanacearum* disease complex: A) tuber galling; B) transverse section of galled tuber infected with *R. solanacearum*.

In surveyed areas, farmers were practicing various management strategies towards *Meloidogyne* spp. and *R. solanacearum*. Out of 180 respondents, 155 (86%) used chemicals, out of 155 respondents, 141 (91%) applied fungicide, 10 (6.4%) applied insecticide, and 4 (2.5%) applied herbicide. Amazingly, none of the respondents used and/or were aware of nematicides and bactericides. Other management approaches were through cultural practices. All respondents (100%) practiced weeding with rather good success across the surveyed areas. Ploughing 132 (73%), crop rotation 117 (65%), host/infected plant eradication 22 (12%), fallowing 3 (2%), and combinations of two or more approaches or integrated disease management 166 (92%) were the remaining strategies.

DISCUSSION

Results of the identification of *Meloidogyne* spp. were based on the morphological characters of perineal patterns of females extracted from infected tubers and soil bioassay tests on soil collected from all surveyed areas and showed the species were *M. incognita* and *M. javanica*. Although only a few samples were identified molecularly, all results supported the identification of *Meloidogyne* spp. by morphological method.

The results revealed that the majority (> 68%) of the samples from soil and tubers were infested with both *Meloidogyne* spp. and *R. solanacearum*. The prevalence and incidence of the two pathogens suggest their importance as a potential threat to potato production in the surveyed areas; thus, management of these pests should be given due attention. The distribution of the diseases and the two disease complexes were uneven and it was highest in Kersa district (1942-2075 m a.s.l). The highest prevalence and incidence of the diseases in the Kersa district might be due to intensive vegetable cultivation and the elevations of the district lower in altitude than other districts. Seid *et al.* (2019) reported that nematode diversity is greater at lower altitudes than higher altitudes. Other reasons for the variation are probably due to factors related to differences in edaphic and climatic factors of the surveyed areas, quality of seeds, or planting materials that were from susceptible potato cultivars. The agro-climatic conditions of the areas, soil type and moisture are reported to affect the population of such soil-borne pathogens (Wallace, 1969; Davide, 1985; Sasser & Carter, 1985; Waals *et al.*, 2013; Ami *et al.*, 2018). Reports indicate that infection of *Meloidogyne* spp. increases with the increase in sand content (Lawrence *et al.*, 1997; Tefera & Hulluka, 2000; Karssen *et al.*, 2013; Kayani *et al.*, 2013; Zia *et al.*, 2014). Seid *et al.* (2019) also reported greater *Meloidogyne* spp. damage in sandy soil type in tomato fields.

The prevalence and incidence of *Meloidogyne* spp. and *R. solanacearum* diseases complex on potato have not been reported in Ethiopia, yet. However, the prevalence of *Meloidogyne* spp. and *Fusarium* wilt (*Fusarium oxysporum*) on tomato crops have been reported (Kassie, 2019). Our study revealed the co-occurrence of the two pathogens in both a single host and soil, and synergistic interaction between the two pathogens in eastern Ethiopia. The synergistic interaction between *Meloidogyne* spp. and *R. solanacearum* on a variety of hosts, such as brinjal (Reddy *et al.*, 1979; Pavithra *et al.*, 2014), tamarillo (Martin & Nydegger, 1982), potato (Suatmadji, 1986; Ateka *et al.*, 2001; Getu *et al.*, 2021), tomato (Sundaresh *et al.*, 2017), has been reported previously. Information on the co-occurrence of the two pathogens in all of the surveyed districts revealed the importance of the pathogens on potato. More research and development work are required to counteract the pathogens.

In line with the present work, the occurrence of the two predominant tropical *Meloidogyne* spp., *M. incognita* and *M. javanica*, has earlier been reported on tomato in Ethiopia (Tefera & Hulluka, 2000; Mandefro & Mekete, 2002; Kassie, 2019; Miheret *et al.*, 2019; Seid *et al.*, 2019).

The majority of the respondents (64%) used farm-saved seed tubers and seed tubers bought from local markets for planting. The use of non-certified seed might contribute towards the prevalence of *Meloidogyne* spp. and *R. solanacearum* disease complex in the surveyed areas. Certified planting materials that are clean and free from diseases are necessary for the management of the diseases. Mulatu *et al.* (2005) reported that most farmers of eastern Ethiopia grew local potato cultivars, except for some farmers in the vicinity of Haramaya University and farmers who are targeted by NGO seed programmes that have access to improved varieties. Hirpa *et al.* (2010) reported the problems of the seed systems in Ethiopia, which seem to provide seed tubers of insufficient quality, and the unavailability of seed tubers of improved varieties. The majority (86%) of the respondants applied chemicals such as fungicide, insecticide and herbicide in order to manage their fields. However, none of the respondents used and/or were aware of nematicides and bactericides. This might be due to unavailability of the chemicals in the nearby areas. However, the chemicals are registered for use in the country (Mengistie, 2016).

ACKNOWLEDGEMENTS

This work is part of a Ph.D. dissertation of the first author. The authors are thankful to the Ministry of Science and Higher Education (MoSHE) and Haramaya University (HU) for their extended material, technical and financial support in carrying out this study.

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T. Getu, W. Mohammed, A. Seid, T. Mekete, B. Kassa and M. Bogale. Распространенность, встречаемость и характеристика комплекса болезней с участием *Ralstonia solanacearum* и *Meloidogyne* spp. на картофеле (*Solanum tuberosum* L.) в восточной Эфиопии.

Резюме. Было проведено исследование для оценки распространенности, заболеваемости и встречаемости комплекса болезней *Meloidogyne* spp. и *Ralstonia solanacearum* (RS) на картофеле. Всего с 92 полей было собрано 92 клубня и 92 образца почвы. Два вида галловых нематод, *Meloidogyne incognita* (MI) и *M. javanica* (MJ), и бактериальный изолят RS были идентифицированы отдельно или в смешанных популяциях. Самая высокая распространенность *Meloidogyn*e spp. и комплекса болезней с участием RS составили 87% в Керсе. Наибольшая заболеваемость комплексом болезней составила 70% в Керсе и Чиро. В образцах клубней встречаемость комплекса заболеваний MI, MJ и RS достигла наивысшего среднего значения 84,6% в Керсе. Осведомленность фермеров об этих патогенах кажется хорошей. Исследование подтвердило одновременное появление *Meloidogyne* spp. и RS у одного хозяина, что указывает на необходимость изучения важности этих патогенов.