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Prevalence and Distribution of Rice Root Knot Nematode in Rice Nurseries in Chitwan and Lamjung Districts of Nepal

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ABSTRACT

To determine the natural infection of the rice root-knot nematode (*Meloidogyne graminicola* Golden & Brichfield) in rice nurseries, a survey was carried out in Chitwan and Lamjung in June and July of 2022. Rice nurseries were randomly selected based on geographical location, agro-ecological region, land type, cropping practice, and soil type. Forty rice nurseries were surveyed i.e. twenty from each district and 100 seedlings from each nursery as the sample for the study using the simple random method of sampling were evaluated. Field survey revealed that *M. graminicola* was widely distributed in most rice growing areas of Chitwan and Lamjung districts. Most of the rice nurseries in Lamjung district were found to be infested with *M. graminicola* as compared to that of the Chitwan district. Disease severity was reported up to 23.26% in Lamjung and 20.2% in Chitwan district. Additionally, the study showed that dry bed conditions were more likely to be affected by rice root-knot disease than wet bed conditions. The majority of farmers planted seedlings on upland (dry) soil, where second-stage juvenile (J2) populations and rice root-knot disease were more common. Most farmers (87.5%) have limited knowledge about rice root knot nematodes and their control methods. This suggested a significant loss in rice output and a high danger of nematode multiplication. This information will provide knowledge about the prevalence of nematode and enabling rice growers in this region to design and implement an appropriate control strategy for managing *M. graminicola*.

Keywords: Galls, Juveniles, *Meloidogyne*, Severity, Upland

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INTRODUCTION

Rice stands as a vital staple for over 3.5 billion people globally (CGIAR 2016). Emerging as the foremost staple crop, it thrives in a wide array of agro-ecological zones, encompassing diverse climates, altitudes, and topography. This includes areas as low as 60 meters in Terai and as high as 3050 meters above sea level in Chumchure, Jumla (Bhujel 2004, Bista 2004). Terai region, hilly region and Himalayan region of Nepal consists of 34,019, 61,345, 51,817 square kilometer area respectively. In Lamjung district, maize is cultivated in an area of 14,332 ha producing up to 48,260 mt with the productivity of 3.37 mt ha^{-1} and it is cultivated in area of 28,457ha producing up to 113,376 mt with the productivity of 3.98 mt ha^{-1} in Chitwan (MoALD 2020).

In rice farming, more than 200 species of plant parasitic nematodes (PPNs) have been reported (Prot 1994). According to Coyne and Plowright (2000), nematodes pose significant challenges within the dynamic rice production system. Among the crucial PPNs associated with rice-based cropping systems, including rice root knot nematodes (*Meloidogyne* spp.), rice root nematode (*Hirschmanniella oryzae*), white tip nematode (*Aphlenchoides besseyi*), and stem nematode (*Ditylenchus angustus*), rice root knot nematodes (*Meloidogyne* spp.) are regarded as a major concern in rainfed, upland, and lowland rice fields. Conversely, rice root nematodes (*Hirchmanniella* spp.) pose problems in lowland rice cultivation regions of South and Southeast Asia (Prot 1994).

As of now, Nepal has encountered three primary rice diseases attributed to plant-parasitic nematodes (PPNs). These include rice root knot nematode, rice root nematode, and white tip disease of rice (*Aphelenchoides besseyi*). According to findings by Pokharel (2007), *M. graminicola* exclusively emerged as the root knot nematode species discovered in a comprehensive survey across 33 rice-wheat farms in Nepal, spanning from the Himalayan region to the Terai. Moreover, *Hirschmanniella oryzae* and *H. mucronata* were the prevalent nematode species identified in a significant portion of rice samples examined in Bhairahawa and Chitwan regions (Pokharel et al 2004), emphasizing their presence in rice fields in these areas. From 1972 to 1974, white tip disease was an issue in the Kathmandu valley, especially among Taiwanese varieties (Shah 1975).

Rice root knot nematodes, specifically *Meloidogyne* spp., are widely recognized as the most significant pests affecting rice fields, including rainfed, upland, and lowland rice cultivation. Of these nematodes, *M. graminicola* is the most problematic in upland regions, feeding on both wheat and rice and causing losses in lowland and deepwater rice farming as well as nursery crops. (MacGowan and Langdon 1989, Bridge et al 1990). Comprehensive study by Pokharel and Sharma-Poudyal (2001) found significant *M. graminicola* populations in the rice fields in Chitwan, Rupendehi, Bara, Parsa, and Rautahat districts of Nepal. Additionally, they noted the presence of the rice root knot nematode in rice and wheat fields that were part of a rice-wheat cropping system, particularly in the districts of Bara, Parsa, and Rautahat. Given that *M. graminicola* may infect the majority of rice types used in Nepal (Pokharel et al 2007, Sharma-Poudyal et al 2004), rice yield losses are probably frequent in all *M. graminicola* infested nurseries and fields. Therefore, research on the root knot nematode infestation and workable nematode control strategies for Nepali farmers is desperately needed. Therefore, the purpose of this study was to determine the extent of *M. graminicola* infection in rice nurseries in the districts of Lamjung and Chitwan.

MATERIALS AND METHODS

In two districts, Chitwan and Lamjung, a random inspection of rice nurseries was conducted in June – July 2021, to check for root knot nematode infestations in rice crops. The Chitwan district is situated between 27°21’45’’ to 27°52’30’’ north latitude and 83°54’45’’ to 84°48’15’’ east longitude, approximately 139 km southwest of the capital Kathmandu. The elevation in the area ranges from 144 to 1947 meters above sea level. The climate in Chitwan valley is subtropical and tropical, characterized by hot and humid summers and cool, dry winters. Moving on to the Lamjung District, it is located between 28°03'30"N to 28°30'00"N latitude and 83°59'00"E to 84°38'00"E longitude, with an altitude of 610 m. This district falls within the sub-tropical to temperate climatic zone of Nepal, experiencing warm and humid summers and cool, dry winters. The average annual rainfall is 203 mm.

The survey was carried out in Khairahani Municipality (Khairahani, Mainaha) and Bharatpur Municipality (Patihani, Rampur) in Chitwan and Besisahar Municipality (Chiti, Besisahar) and Sundarbazar (Sundarbazar, Parewadada) Municipality in Lamjung from June to July 2022. The selection of the study area was made based on consultation with Agriculture Knowledge Centre, Chitwan and Lamjung and review of past works. Two different districts were selected to compare the nematode distribution and incidence in two different ecological regions. Ten nurseries were surveyed in each municipality and from each of the districts a total of 20 samples were collected. Information such as the name of the farmer, location, days of seed sowing, land type (lowland or upland), seed bed type, soil type, variety of seed, and previous crop sown were taken.

Rice roots were pulled randomly in 'M' fashion with the soil intact at each nursery site, and samples were gathered from 5 positions, with a total of 20 plants from each nursery. Sampling from the rice nursery involved uprooting the entire plant from the soil using a spade. Care was taken to extract the entire root system without causing any damage to the roots. The plants were then labeled, placed into a paper bag, and delivered to the Plant Pathology Laboratory at Agriculture and Forestry University (AFU) in Chitwan. Samples were stored at 4°C until nematode extraction.

In the lab, adherent soils were carefully removed, and a variety of observations were made, including the number of infected plants, and the length of the roots and shoots of infected and non-infected plants. A modified Bayermann tray was used for the extraction process, and about 2 g of chopped roots were combined with 100 ml of water in an electric blender and mixed for 2 minutes (Schindler 1961). The nematode suspension was collected in 50 ml plastic tubes during a 48-hour processing period. Using a glass pipette, the final volume of suspension was adjusted to 20 ml after an hour of standing. After allowing the 20 ml suspension in the counting disk to settle for five minutes, a two-milliliter (10%) aliquot was taken. J2 were counted while being viewed with a stereo microscope (Bridge et al 2000). Perineal patterns of five adult females per sample were used to identify the root knot nematode.

Root knot index was calculated, using (0-10 scale) developed by Bridge et al. (2005). Root knot severity index was determined as follows:

$$\text{Root knot severity index} = \frac{\text{Sum of all numerical rating}}{\text{Total number of rating} \times \text{Maximum grade}} \times 100$$

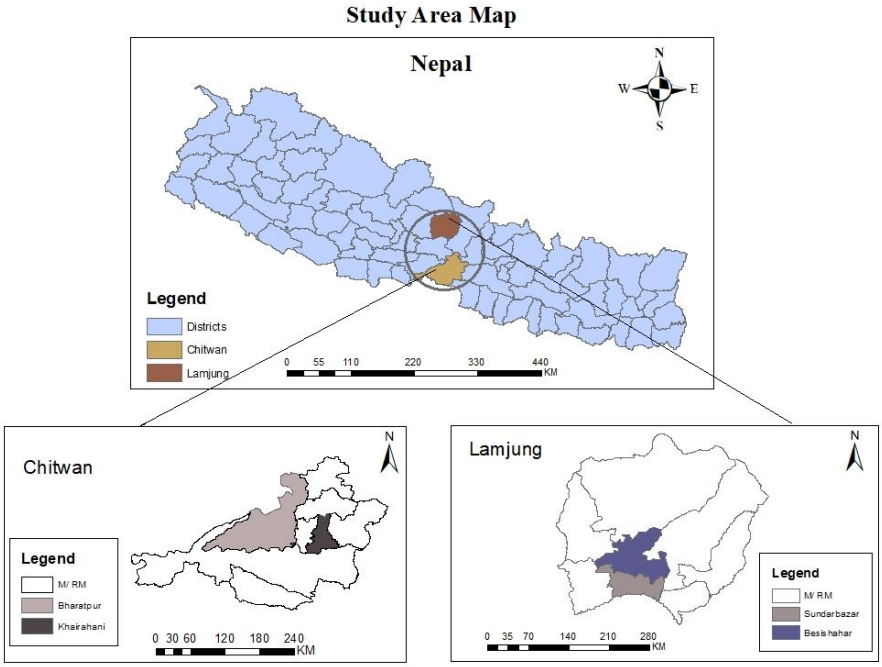

Figure 1. District map of Nepal showing study site

RESULTS

According to the study results, the majority of farmers (52.5%) cultivate rice plants on fallow land, and around 25% of them plant after maize. Others grew after rice, mustard, onions, and cucurbits. 'Sabitri' was the most popular variety among farmers in Chitwan (45%), followed by Owar (Ram Dhan) (20%) and Sawa Mansuli (15%). Sabitri, Sona Mansuli, and Jyoti were the other cultivars cultivated. Additionally, in Lamjung, of the rice nurseries surveyed, 'Ram Dhan' (30%) was the most cultivated variety, followed by Mansuli (15%). Sukkhadhan-2, Buchhe, Sona Mansuli, Eksare, Ekle, Jhinuwa, and Aanadi were additional cultivars of rice that were cultivated. None of the farmers treated soil or seeds with pesticides, some of them utilized techniques like saltwater seed priming. In comparison to galled plants, non-galled plants had much longer shoots and a larger overall plant height. In various nurseries, the percentage of galled seedlings ranged from 0% to 91%. Up to 23.26% of root knots were considered severe. Up to 59 *M. graminicola* juveniles (J2s) were recovered from 2 grams of rice root.

In the investigated rice nursery beds, the frequency of root knot nematode was found to be 50% in Chitwan and 55% in Lamjung districts. In overall, the highest root knot severity was recorded in Lamjung district i.e. 10.24%, while it was found to be 6.83% in Chitwan district. (Table 1). In Chitwan and Lamjung districts combined, 32.5% of the farmers grew rice plants in lowland conditions and 67.5% of the farmers grew in upland conditions. Under the upland seedbed, approximately 25.08% of plants were galled with root knot severity index of 7.58 %, and an average number of *M. graminicola* J2 extracted from 2 g root was recorded up to 20.07. In lowland conditions, 3.71% of rice plants were galled and root knot severity index was recorded as up to 1.05%, and an average number of *M. graminicola* J2 extracted from 2 g root was observed up to 3.83 as shown in Table 2.

About 20% of the farmers selected grew rice in wet beds, whereas the remaining 80% produced it on dry beds. In comparison to the dry bed, the wet bed had a much lower galled plant percentage and a lower root knot severity index. In a dry bed, the root knot severity index is 7.65%, whereas in a moist bed, it is 1.25%. Comparably, 8.25% and 18.41%, respectively, of galled plants are found in wet and dry beds.

Table 1. Average frequency of occurrence, galled seedlings, disease severity and J2 population of *Meloidogyne graminicola* in nursery beds of rice in Lamjung and Chitwan districts of Nepal, 2022.

Districts	Municipality	Frequency of occurrence (%)	Galled seedlings (%)	Disease severity (%)	J2 population per 2g of root
Chitwan	Bharatpur	60	42.1	11.29	22.9
	Khairahani	40	5.9	2.38	7.8
	Total	**50**	**24**	**6.83**	**15.2**
Lamjung	Besishahar	70	45.4	14.386	25.2
	Sundarbazar	40	10.01	7.111	12.9
	Total	**55**	**27.75**	**10.24**	**19.05**

Table 2. A comparison of the rice root-knot disease development in lowland, upland, dry bed, and wet bed conditions.

S.N	Parameters	Upland	Lowland	Dry bed	Wet bed
1.	Root galling percentage	25.038%	3.71%	18.41%	8.25%
2.	Root knot severity index	7.579 %	1.05%,	7.65%	1.25%
3.	J2 population per 2g of root	20.071	3.833	15.48	5.14

Most of the farmers surveyed use farm yard manures (65%) in their rice nursery beds and 30% of them do not use any kind of fertilizer in their seed bed. Another type of fertilizer source used by farmers in the rice seedbed was chicken manure and urea.

DISCUSSION

Four locations in Chitwan and Lamjung were investigated for the existence of rice root knot nematode: Khairahani, Rampur, Patihani, and Mainaha in Chitwan, and Chiti, Besishahar, Parewadada, and Sundarbazar in Lamjung. According to the study, both areas had infestations of rice root knot nematodes. However, in the nursery beds that were examined, Lamjung had a greater incidence of root knot nematode than Chitwan. Rampur and Patihani in Chitwan have the greatest levels of root knot nematode as presented in Table 1. The findings of Pokharel and Sharma-Poudyal (2001), who also found high levels of *M. graminicola* in rice fields held by farmers in many districts of Nepal, including Chitwan, Rupendehi, Bara, Parsa, and Rautahat, are consistent with the findings reported here. Similar to this, Chiti and Besisahar in Lamjung had the highest prevalence of the root knot nematode. Other studies, including those by Dangal et al (2009), Pokharel (2009) and Pokhrel et al (1997) have additionally confirmed the presence of *M. graminicola* in rice nursery fields in both Lamjung and Chitwan. According to result presented on Table 2, *M. graminicola* infestation in Chitwan and Lamjung districts, it was noticed that upland conditions had a larger percentage of galled plants and a higher root knot severity index than lowland conditions. Soriano and Reversat (2003) also reported that the aerobic upland systems has more damage from *M. graminicola* than lowland conditions. In contrast, rice roots had a greater chance of fending off *M. graminicola* invasion in lowland regions with ongoing flooding (Bridge and Page 1982). Furthermore, frequent flooding in lowland environments may make rice plants more resistant to *M. graminicola* (Soriano et al 2000). The majority of rice production in Chitwan has lowland conditions as compared to that of Lamjung district which has the majority of rice fields in upland conditions. So due to this reason, there might be a higher occurrence and disease infestation of rice root-knot nematode in Lamjung as compared to that Chitwan district.When compared to wet seed beds, dry seed beds were shown to have a higher incidence of *M. graminicola*. The values for the root knot severity index (%) and the percentage of galled plants in the dry beds were much greater than they were in the moist beds. The rice roots were able to escape *M. graminicola* invasion in the wet beds, which were regularly watered to keep them consistently saturated (Bridge and Page 1982), and this may have restricted the spread of nematode (Prot and Matias 1995). Plant height and root length surged as a result of the lower infection rates seen under moist bed condition. Furthermore, the amount of J2 extracted from 2g of the root, the galled plants percentage, and the root knot severity index (%) were all lower in the wet bed conditions than in the dry bed. This shows that the wet bed condition was adverse to *M. graminicola* infection and growth. Due to inadequate respiration and nematode movement brought on by reduced aeration brought on by persistently excessive moisture levels in the wet beds, *M. graminicola* population dwindled (Garg et al 1995)

CONCLUSIONS

The survey results showed that the rice root nematode, *M. graminicola*, is infesting rice nurseries in both Chitwan and Lamjung districts, which are important rice-growing regions in the country. The nematode infestation was found to be more severe in Lamjung compared to Chitwan district. Various ecological conditions favored the infestation, such as nursery type, seed treatment, previous crop sown and variety/cultivar, and other cultural practices. This could be attributed to the lack of control measures being used by farmers in

these areas. The high density and frequent occurrence of the nematode population in rice nursery indicate a lack of success in nematode management in both districts. These findings are valuable for breeding programs and for extension staff to raise awareness about the presence of root-knot nematodes affecting root knot nematode in rice.

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AUTHORS' CONTRIBUTION
Srijan Tiwari: Performed the survey; Carried out laboratory experiment; Analyzed and interpreted the data; Wrote the manuscript. Hira Kaji Manandhar: Regular constructive comments and support during the research period; Guidance during the preparation of paper. Prem Pandey: Support during the research period; Guidance during the preparation of manuscript. Shyam Sundar Nath: Assisted in manuscript writing and reviewed final draft of manuscript

CONFLICTS OF INTEREST

The authors have no any conflict of interest to disclose.

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