



**Full Length Article**

## Causes of Soil Sickness Associated with Aerobic Rice Continuous Monocropping

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### Abstract

Soil sickness related with yield decreases of continuously monocropped aerobic rice (CMAR) is the most important constraint to the wide implementation of aerobic rice technology. Root knot nematodes are considered to be the most important contributors of soil sickness because of continuous monocropping of aerobic rice, followed by fungi, and other micro-organisms. The main purpose of this study was to investigate the biotic factors responsible for the soil sickness due to CMAR. Two experiments with split-pot arrangement were carried out using soil from field under aerobic rice for continuously eleven growing seasons and a gradual yield decline has been already reported. In both trials, one half of pot was filled with oven-heated sick soil and other half with untreated sick soil. The oven-heated sick soil was separated from untreated sick soil by two different holders i.e., with nylon filter (0.2  $\mu\text{m}$  pore size) or steel net (1.0 mm pore size). Apo cultivar was aerobically grown without fertilizer inputs in both experiments. No difference was found for growth traits of Apo cultivar grown with oven-heated soil between the pots with two different holders in experiment 1. In experiment 2, the grain yield and aboveground biomass of Apo were even lower in the oven-heated soil of pots using holders with 0.2- $\mu\text{m}$ -pore-size nylon filter than those with steel net. Moreover, there was no difference in root-knot nematode population of plants grown in untreated soil between the pots with two different holders. These results suggest that biotic factors are impossible to create soil sickness related with CMAR. Identifying the causes responsible for soil sickness and then developing mitigation strategies are vital for sustainable production of aerobic rice. © 2014 Friends Science Publishers

**Keywords:** Biotic factors; Monocropping of aerobic rice; Nylon filter; Soil sickness

### Introduction

Aerobic rice is a production system in which high-yielding rice is grown in non-puddled and unsaturated soil under non-flooded conditions. The system requires reduced inputs, can be rain dependent or irrigated and occasionally flooded (Bouman and Tuong, 2001). According to Bouman *et al.* (2005), aerobic rice saves water use by 27-51% and increases water productivity by 32-88%. Furthermore, the labor is conserved and greenhouse gas emission is reduced in aerobic rice compared with irrigated rice (Wang *et al.*, 2002). With proper cultivars in aerobic rice system, the grain yields of above 5 t ha<sup>-1</sup> are obtained (Bouman *et al.*, 2006; Peng *et al.*, 2006).

However, yield decrease of CMAR has been reported by many researchers, which is a key restriction to the well-known implementation of aerobic rice technology (Nishizawa *et al.*, 1971; Ventura and Watanabe, 1978; Guimaraes and Stone, 2000; Fageria and Baligar, 2003; Kreye *et al.*, 2009). It is generally considered that inhibition of growth and yield decline of CMAR was caused by soil

sickness (Nishizawa *et al.*, 1971). However, the causes of soil sickness related with monocropped aerobic rice have not yet been identified. Some scientists are of the view that soil sickness in aerobic rice is mainly attributed to biotic factors; and nematodes are usually considered to be the most important contestant among biotic factors, followed by fungi, and other micro-organisms (Watanabe and Yasuo, 1960; Nishizawa *et al.*, 1971; Nishio and Kusano, 1973; Kreye *et al.*, 2009). The purpose of this experiment was to investigate if these biotic factors are responsible for the soil sickness due to CMAR.

### Materials and Methods

#### Soil Description

Two experiments with split-pot arrangements were carried out in the greenhouse at International Rice Research Institute (IRRI). For both experiments, soil was collected from field under aerobic rice continuously for 11 growing seasons since 2001 dry season and for which a gradual

decrease in yield has been already reported (Peng *et al.*, 2006). The soil physical and chemical properties were presented in Sasaki *et al.* (2010).

### Design of the Split-pot Experiments

The pots used for these trials were made up of polymethyl methacrylate with dimensions of 20 cm (length) × 20 cm (width) × 30 cm (height). One half of the pot was filled with 4.0 kg of oven-heated sick soil, while the other half with the same weight of untreated sick soil. Oven heating has been adopted as one of the soil sterilization methods to lighten soil sickness produced by CMAR by previous researches (Nie *et al.*, 2007, 2008, 2009a; Sasaki *et al.*, 2010). According to Nie *et al.* (2007) biotic factors related to soil sickness can be removed by soil oven heating. Oven-heated sick soil was separated from untreated sick soil by holders with nylon filter (0.2 μm pore size, Gelman Sciences Inc.) or steel net (1.0 mm pore size). Holders were inserted into the pots and divided into two equal parts (Fig. 1). For soil heating treatment, the air dried up soil was put in an oven and autoclaved up to 120°C for 12 h (Sasaki *et al.*, 2010).

The 0.2 μm pore-size nylon filter does not allow micro-organisms including nematodes, fungi, and bacteria from passing through because of their greater size than 0.2 μm (Rosenberg, 1979). Thus, in these two experiments, the biotic factors could pass from the untreated sick soil side to oven-heated soil side through steel net, but not through nylon filter, while nutrients could flow across both steel net and nylon filter. In both experiments, an upland rice variety, Apo was used. The seeds were soaked with tap water for 24 h and pre-germinated in a constant incubator for 24 h before sowing.

In experiment 1, six seeds were sown in oven-heated soil, while no seed was sown in untreated soil. In experiment 2, six seeds were seeded in both the oven-heated soil and the untreated soil. Three replications were made for each treatment. In order to maintain three consistent numbers of seedlings on each side, one week after sowing thinning was conducted. Plants were grown aerobically without any nutrient supply. Every time, water was irrigated from untreated soil side. Experiment 1 was started on 20<sup>th</sup> June 2006 and plants were sampled on 7<sup>th</sup> August 2006. In experiment 2, the seeding was done on 1<sup>st</sup> February 2007 and the plants were harvested at maturity.

### Plant Growth Analysis

Before sampling, height of the plant was precisely measured and stem number of each pot was counted. After harvesting, stems, leaves, and roots in experiment 1 were separated from plants while straw, panicles, and roots in experiment 2. In experiment 1, measurement of leaf area was carried out by using the Licor 3100. For dry weight, samples were put in an oven at 70°C until constant weight. Total biomass was

computed by adding stem, leaf and root dry weights in experiment 1. In experiment 2, spikelets number per panicle, grain-filling percentage, aboveground biomass and harvest index were calculated.

### Nematode Population Assessment

Roots nematodial population was examined by collecting intact roots. First roots were dig-out from soil and then washed by using running water in order to clean the soil from roots. After cleaning roots were cut into 1 cm lengths and put it in the mistifier for 14 d (Seinhorst, 1950). Nematode juveniles were identified after 7 and 14 d and counted by using stereoscopic microscopes. Fresh and dry weights of the root samples for nematode evaluation were recorded.

### Statistical Analysis

Data were statistically analyzed using analysis of variance (SAS Institute, 2003). The means were separated using Least Significance Difference (LSD) at 5% probability level.

### Results

No differences were observed between the pots with two different holders in terms of plant height, stem number, leaf area, root biomass, and total biomass (Table 1). The result of nematode evaluation demonstrated that nematode inhabitant was 1.4 g<sup>-1</sup> root of plants grown in oven-heated sick soil using holder with steel net, however no nematode infestation was observed in roots of plants grown in oven-heated sick soil using holder with 0.2-μm-pore-size nylon filter. This was consistent with an earlier report that 0.2-μm-pore-size filter can stop micro-organisms including nematodes, fungi, and bacteria from passing through.

No significant difference was observed while comparing the plant growth, grain yield and its components of Apo grown in the oven-heated soil between the pots with two different holders, in terms of plant height, panicle number, spikelets per panicle, harvest index, grain filling percentage and root dry weight (Table 2). On other hand, grain yield, aboveground biomass, and rice root-knot nematode population in the oven-heated soil of pots using holders with 0.2-μm-pore-size nylon filter were significantly lower than those with steel net. For the untreated sick soil, grain yield, panicle number, aboveground biomass, harvest index, and grain filling percentage were significantly higher in pots using holders with 0.2-μm-pore-size nylon filter than those with steel net. There were no differences in plant height, spikelets per panicle, root biomass, and root-knot nematode population of plants grown in the untreated soil between the pots with two different holders.

The results from experiment 1 suggested that biotic factors did not cause the soil sickness related to CMAR.

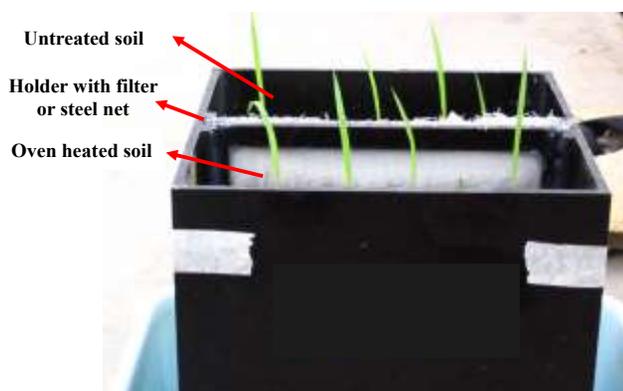
**Table1:** Plant growth and root nematode population of Apo aerobically grown with oven heated soil of experiment 1

Parameter	Holder with filter	Holder with steel net
Plant height (cm)	82.70 a	82.70 a
Stem number per pot	32.30 a	33.30 a
Leaf area (cm <sup>2</sup> pot <sup>-1</sup> )	2530 a	2716 a
Root dry weight (g pot <sup>-1</sup> )	3.80 a	4.10 a
Total biomass (g pot <sup>-1</sup> )	25.80 a	27.40 a
Rice root-knot nematode (no. g <sup>-1</sup> root)	0.00 b	1.40 a

**Table2:** Plant growth, grain yield, yield components and root nematode population of Apo aerobically grown with oven heated and untreated soil of experiment 2

Parameter	Holder with filter		Holder with steel net	
	Oven heated soil side	Untreated soil side	Oven heated soil side	Untreated soil side
Plant height (cm)	92.00 a	79.30 b	95.20 a	80.40 b
Panicle number per side	18.70 a	8.30 b	21.10 a	3.20 c
Spikelet per panicle	104.20 a	83.10 b	103.90 a	88.30 b
Aboveground biomass (g side <sup>-1</sup> )	68.46 b	21.44 c	81.01 a	8.82 d
Harvest index	0.34 b	0.43 a	0.35 b	0.31 b
Grain yield (g side <sup>-1</sup> )	23.23 b	9.16 c	28.35 a	2.76 d
Grain filling	71.70 b	81.50 a	71.80 b	60.00 c
Root dry weight (g side <sup>-1</sup> )	9.70 a	2.81 b	9.07 a	2.15 b
Rice root-knot nematode (no. g <sup>-1</sup> root)	0.00 c	6.80 a	3.10 b	5.90 a

† Within a row, means followed by different letters are significantly different at 0.05 probability level according to Least Significant Difference (LSD) test


**Fig. 1:** Illustration of the split-pot experiment. The port size of nylon filter and steel net are 0.2 μm and 1.0 mm, respectively

**Fig. 2:** Aboveground (left) and root (right) growth of Apo aerobically grown on the side with oven heated soil in split-pot experiment 1. The port sizes of nylon filter and steel net are 0.2 μm and 1.0 mm, respectively

Although the biotic factors (e.g., nematodes) can pass from

the untreated sick soil side to oven-heated soil side through steel net, the differences in plant growth between the pots with two different holders were not observed (Table 1). While from experiment 2, results were not consistent with experiment 1. The grain yield and aboveground biomass of the plants developed in the oven-heated soil side were significantly lower in pots using holders with 0.2-μm-pore-size nylon filter than those with steel net, although there was no nematode infestation in the roots grown in the oven-heated soil with 0.2-μm-pore-size nylon filter (Table 2). Such findings further confirm that biotic factors may not be the main factors responsible for the soil sickness due to the continuous aerobic rice monocropping.

## Discussion

Two cases explained the differences in plant growth performance of Apo between two experiments. First, plants in experiment 1 were sampled at 48 d after sowing, while for experiment 2 plants were harvested at maturity stage. Second, plants were grown aerobically without any nutrient supply. It can be suggested that as the plants further grew, more nutrient and water requirements forced the roots grown in the oven-heated soil to penetrate across the steel net to the untreated soil, but roots could not drill through the 0.2-μm-pore-size nylon filter. This assumption is also supported by the fact that grain yield, panicle number, aboveground biomass, harvest index and grain filling percentage of plants grown in untreated sick soil were significantly lower for the pots having holders with steel net than those with 0.2-μm-pore-size nylon filter (Table 2).

Ventura *et al.* (1981) documented that nematodes were not related with soil sickness of aerobic rice at IRRI farm. In

our both experiments, there were no differences in plant growth parameters between plants with and without infestation of rice root-knot nematode. Furthermore, under continuous aerobic rice cultivation, population of nematodes was not found completely high and did not increase significantly along with increased number of seasons (Nie et al., 2009b). Therefore, nematodes may not cause the soil sickness due to CMAR in this study. Among biotic factors, only nematode population was examined in this research. That is because nematodes are usually deemed to be the prime consideration among biotic factors. Furthermore, in our relative research, we did not observe significant differences in fungi, bacteria, actinomycetes, and algae population between the 10<sup>th</sup> season and 1<sup>st</sup> season aerobic rice soils (data not shown).

From this study it is difficult to understand whether biotic or abiotic factors were relevant to soil sickness. However, the results suggest that biotic factors are less likely to cause soil sickness associated with CMAR. This research on causes about soil sickness due to CMAR was carried out at IRRI farm and the results could be site-specific. The exact causes of the soil sickness resulting from continuous aerobic rice system are still not recognized. Identifying the causes responsible for the soil sickness and developing mitigation strategies are some of the prerequisites to realize sustainability of aerobic rice production.

Conclusively, the results suggest that biotic factors are not responsible to cause soil sickness associated with continuous monocropping of aerobic rice. However, the results may be site-specific and require environmental replication using different soils.

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