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# Effect of Nutrient Management and Weeds on Incidence of Fungal Diseases in Rice

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## Abstract

High-input, modern agriculture uses large amounts of energy, water, fertilizers, and pesticides to produce high crop yields. One of the major bottlenecks of the modern agriculture in the tropics is substantial yield losses due to fungal diseases including rice blast, leaf spots and leaf scald. The aim of this study was to compare the incidence of fungal diseases in judicious nutrient management systems, including organic, integrated, and conventional, under different weed categories during dry season (May to September 2020) and wet season (November 2020 to March 2021). Rice disease incidence were collected for both seasons from 48-84 days after sowing. Additionally, disease incidences on grasses and sedges weeds were also calculated. Brown spot, narrow brown leaf spot, leaf scald, and rice blast incidences were substantial in wet season, while the disease incidences during dry season in 2020 were negligible. The disease incidences were significantly higher (*P<0.05*) in organic and conventional input systems compared to the integrated input system. Disease incidences of brown spot and leaf scald were found in the dry season. Higher disease incidences were recorded in the wet season than in the dry season. The incidences of the brown spot were higher on sedges than in grasses and vice versa were observed for narrow brown leaf spot disease. Leaf scald incidences were positively correlated with the significant nitrogen status of the rice crop. Disease incidence was low in integrated input system compared to conventional and organic input systems, while weeds were reported as alternative hosts. It can be concluded that the integrated nutrient management with recommended dosage of nitrogen application with proper weed management can lead to low disease incidents, hence is ecologically more sustainable.

Keywords: brown spot, narrow brown leaf spot, nutrient management, rice, weed management.

## Introduction

The major challenges of rice cultivations include insect pests, diseases, and weeds. Microbial pathogens like fungi, viruses, and bacteria are responsible for diseases like rice blast, rice yellow mottle virus disease, and bacterial blight on rice (Skamnioti and Gurr, 2009). According to IRRI (year) rice pests and diseases caused around 37% of yield losses in rice, whereas in the most severe condition, yield losses due to fungal disease can reach 80% (Godfray et al., 2016). However, depending on the production environment, these yield losses can vary between 24% - 41% (Sparks et al., 2012). Weeds may become alternative host habitats to disease-causing pathogens (Seneviratne and Jeyanandarajah, 2004). Therefore, effective fungal rice disease control strategies are essential to reduce yield losses.

During the past few decades, synthetic chemicals have been used to control rice diseases (Khoury and Makkouk, 2010). Unfortunately, chemical disease control in agriculture has resulted in numerous hazardous effects, including environmental pollution, fungal resurgence, the emergence of resistant fungal strains, and secondary fungal infections (Singh et al., 2021). Therefore, research scientists have started focusing on more efficient and environment-friendly disease control in rice cultivations (Zibaee, 2013). A need for strategic studies on disease management in rice has already been stated. Nutrient-based disease management approaches are now evolving to meet this research needs (Walters and Bingham, 2007).

Plant diseases can be controlled by, among others, managing the supply of mineral nutrients to plants at the appropriate time, while also reducing pesticide application. Optimal nutrition management for crops can enhance plant health and resistance to diseases. Minerals including nitrogen (N), sulphur (S), phosphorus (P), potassium (K), and silicon (Si) directly affect pathogens and plant resistance or tolerance against pathogens (Walters and Bingham, 2007). Nitrogen is essential for plant growth and development, and plants obtain it from the soil in various forms such as nitrate, ammonium, and urea. Managing the supply of nitrogen to crops is crucial for their health; excessive use of nitrogen fertilizers can lead to environmental issues. N levels in plants has been correlated to high infections and sporulation of fungal pathogens (Walters and Bingham, 2007). Either high or low N may result in significantly higher foliar fungal diseases incidences (Robert et al., 2002); high N fertilizer inputs increases the incidence of foliar diseases narrow brown leaf spot (Simón et al., 2003; Walters and Bingham, 2007). High N in leaf tissues creates a favourable environment for fungal disease development.

In low external input systems, such as organically grown crops, little knowledge is available on the relationship between nutrient management and disease development. In organic systems, a limited supply of nitrogen reduces the $K^+$ utilization by the crops and may also limit other nutrient supplies (Fortune et al., 2004). This also causes a reduction in plant tissue concentrations and vigour, which may increase disease development through reducing the plant's immune strength. Therefore, adjusting fertilizers judiciously can minimize disease development and spread (Walters and Bingham, 2007). This concept of integrative plant nutrition management is a key element in a sustainable agriculture. Incorporation of nutrients through amendments or modifications of the soil environment is important for the management of rice plant diseases (Huber and Haneklaus, 2007).

The target of this research was to assess the incidence of fungal rice diseases in organic, integrated, and conventional nutrient management systems in wet and dry seasons, along with disease incidence on weeds and the nitrogen status of the crops.

## Materials and Methods

The field experiments were conducted during the 2020 Srilanka's *Yala* season (dry season; May 2020 to September 2020) and 2020/2021 *Maha* season (wet season; November 2020 to March 2021) in the research field of the Faculty of Agriculture, Rajarata University of Sri Lanka, Puliyankulama, Anuradhapura. The site has an agro-ecological region of DL1b. The study area consisted of imperfectly drained reddish brown earth soils (Dassanayake et al., 2020). Rainfall, relative humidity, and temperature were collected from at the meteorological station, Anuradhapura (Table 1). The laboratory studies were conducted at the Plant Pathology Laboratory, Faculty of Agriculture, Rajarata University of Sri Lanka.

*Experimental Design and Treatments*

Three main nutrient input systems, conventional input system (CONV), integrated input system (INT) and organic (ORG) input systems, were tested in this study. These systems were different from each other based on the sources of N fertilizer applied. CONV uses 100 % inorganic fertilizer application as recommended by the Department of Agriculture (DOA) 2013; INT uses 50% inorganic and 50% organic fertilizers; ORG did not apply inorganic fertilizers and the soil fertility was managed through pre-determined organic manure application at a half rate to equate N application in

Table 1. Treatments and the nutrient content

| | Nutrients | Source ($kg.ha^{-1}$) | Mineral fertilizer rate (DOA 2013) ($kg.ha^{-1}$) | Nutrients from organic fertilizer ($kg.ha^{-1}$) | Organic fertilizer ($kg.ha^{-1}$) |
|---|---|---|---|---|---|
| Conventional nutrient management (CONV) | N | 103.5 (Urea) | 225 (Urea) | 0 | 0 |
| | P | 3.9 ($P_2O_5$) | 55 (TSP) | 0 | 0 |
| | K | 30.0 ($K_2O$) | 60 (MOP) | 0 | 0 |
| Integrated nutrient management (INM) | N | 51.8 (Urea) | 112.5 (Urea) | 25.9 | 6 |
| | P | 1.9 ($P_2O_5$) | 27.5 (TSP) | 41.52 | 6 |
| | K | 15 ($K_2O$) | 30 (MOP) | 52.5 | 6 |
| Organic nutrient management (ONM) | N | 0 | 0 | 51.8 | 12 |
| | P | 0 | 0 | 83.03 | 12 |
| | K | 0 | 0 | 0 | 0 |

Note: Urea contains 46% N, $P_2O_5$ contains 43.7% P, $K_2O$ contains 60% K, MOP contains 62% $K_2O$.

the conventional system. Organic fertilizers were prepared by incorporating buffalo manures, poultry manures, *Gliricidia* and *Crotalaria* leaves, which had the N content of 0.2%. The nutrient management systems were defined based on the elemental N supply and sources as described in Table 2. The phosphorus (P) and potassium (K) rates were not standardized; the amount of these two elements were dependent on the quality of materials used to supply N (Table 2). The three treatments were laid-out as a randomized complete block design with six replicates in dry season and 12 replicates in wet season. The size of a single plot was 90 $m^2$ (15 m × 6 m).

Table 2. Rainfall, temperatures, and relative humidity during 2020 *Yala* (dry) and 2020/2021 *Maha* (wet season) in Srilanka.

| | Yala | Maha |
|---|---|---|
| Mean rainfall (mm) | 94.7 | 150.22 |
| Minimum temperature (°C) | 25.08 | 23.39 |
| Maximum temperature (°C) | 32.70 | 30.82 |
| Relative Humidity (%) | 79.79 | 83.94 |

*Crop Establishment and Management*

Pre-germinated seeds of the “Bg300” rice variety were broadcasted at a rate of 120 $kg.ha^{-1}$ in both wet and dry seasons. The application of inorganic fertilizer was based on the DOA recommendation in 2013. Organic fertilizer was applied with basal dressing and at the third top dressing of inorganic fertilizers. Irrigation was started one week after the seed sowing and a water depth of 5 cm was impounded above the soil level to retain sufficient moisture throughout the cultivation period. The flood irrigation method was practiced, and bund were used to separate each plot to avoid water flow from one to another plot.

*Pest and Weed Management*

The insect pest incidences were low during the crop cycles; thus, agrochemicals were not applied to control insect pests. For both conventional and integrated systems, Pretilachlor (a.i. 2-chloro-2′, 6′-diethyl-N-(2-propoxyethyl) acetanilide) + Safener 300 EC was applied to the plots at a rate of 1.6 $L.ha^{-1}$ three days after crop establishment and 14 days after sowing. Bispyribac sodium was applied as an organic weed controller at a rate of 225 $g.ha^{-1}$. Hand weeding was done to remove visible weeds in the organic input system. Analogous to the herbicide application to conventional and reduced, weeds were thinned out by removing visible weeds during the period of 3 days to 14 days. Thereafter, in the organic system, weeds were not controlled.

*Data Collection*

Quadrats with dimensions of 50 cm × 50 cm were used to collect data. Two quadrat samplings were done for each plot with three days interval between two sampling days. The data were collected for 12 sampling days (commencing from the disease) from 48 days after sowing (DAS) to 84 DAS.

*Disease Incidence*

Plots were allowed for natural rice disease incidence. The number of infected plants and the total number of plants were counted within a quadrat to obtain disease incidence. Data collection for disease incidence was started from 48 DAS. To calculate the disease incidence the following equation was used:

$$\text{Disease Incidence} = \frac{\text{Number of infected plants per quadrat}}{\text{Total number of plants per quadrat}}$$

Accordingly, disease incidence was calculated for brown spot, narrow brown leaf spot, rice blast and leaf scald by observing the symptoms.

Diseased leaf samples with moderately large lesions were randomly collected from the field and were cultured after disinfection with 10% Clorox solution. Standard protocol was followed for the culturing process (Delves-Broughton, 2007). Isolation and purification of the responsible fungal pathogens by using the samples were done at the Plant Pathology laboratory. First, the infected leaves were surface sterilized using 1% sodium hypochlorite solution and 70% ethanol for 1 minute followed by a washing step with distilled water. Potato Dextrose Agar (PDA) medium was used for culturing the microorganisms. Surface sterilized samples were dried after placing between two sterile filter papers. Then the samples were placed on PDA medium under the aseptic conditions. Subsequently, the samples were incubated at 28 ± 1°C for 72 h. Identification of fungi associated with symptoms was done based on spore morphology, cultural characteristics, and microscopic observations.

1. Brown spot disease (BS): *Cochliobolus miyabeanus*
2. Narrow brown leaf spot disease (NBLS): *Cercospora janseana*
3. Rice blast (RB)*: Magnaporthe oryzae*
4. Leaf scald (LS)*: Monographella albescens*

After observing the symptoms related to each disease, the Koch postulation was tested on the samples taken from the field to confirm the disease (Koch, 1891).

*Weed Count*

Total number of weeds categorized into grasses, sedges and broadleaves were taken within a quadrat. Then, the number of disease-infected weeds were also counted to calculate the disease incidence. The diseases were also noted down separately. Quadrat samplings were done randomly at two different locations within each plot. Weed counts were done starting from 48 DAS.

*Leaf Nitrogen Status*

Nitrogen status analysis was conducted on leaf samples collected from a 50 cm × 50 cm quadrant at 40 and 60 DAS. Relative leaf chlorophyll content was measured as SPAD (Soil Plant Analysis Development) value using SPAD-502 Plus- leaf chlorophyll meter (Konica Minolta Sensing Inc., Japan). Total nitrogen content of plant samples was determined by the Kjeldhal procedure (Bremner and Mulvaney, 1982) and extractable chlorophyll content was analyzed using Arnon's method (Arnon, 1949).

*Data Analysis*

The disease incidence variation with seasons, input systems and weed abundance were analysed by ANOVA (Analysis of Variance) using the Repeated MIXED model in SAS statistical software program version 9.0. The treatments (input systems) were considered as fixed factors and the replicates (blocks) were considered as random factors. All means were separated using the LSD method at 5% probability level. The correlation between disease incidence and foliar nitrogen content was assessed using the Pearson correlation coefficient at $p<0.05$.

## Results and discussion

*Effect of Seasons on Disease Incidences*

Fungal disease incidences of narrow brown leaf spot (NBLS), brown spot (BS), rice blast (RB) and leaf scald (LS) were observed in the rice field during wet season of 2020/2021. Incidences of BS and LS disease were limited to dry season of 2020. Disease incidences of BS (P<0.0001), NBLS (P<0.0001) and RB (P<0.0001) were significantly different between the dry season (May 2020 - September 2020) and the wet season (November 2020 - March 2021), except for LS (P=0.06). The disease incidences were higher in the wet season than in dry season. The seasonal differences were limited to BS and LS, while NBLS and RB were recorded only in the wet season (Figure 1). Wet season is more humid than the dry season. In comparison, the amount, frequency, and incidences of precipitation are higher in wet season, than in the dry season (Table 1). The biological cycles of pathogens are affected by climatic changes (McMichael et al.,

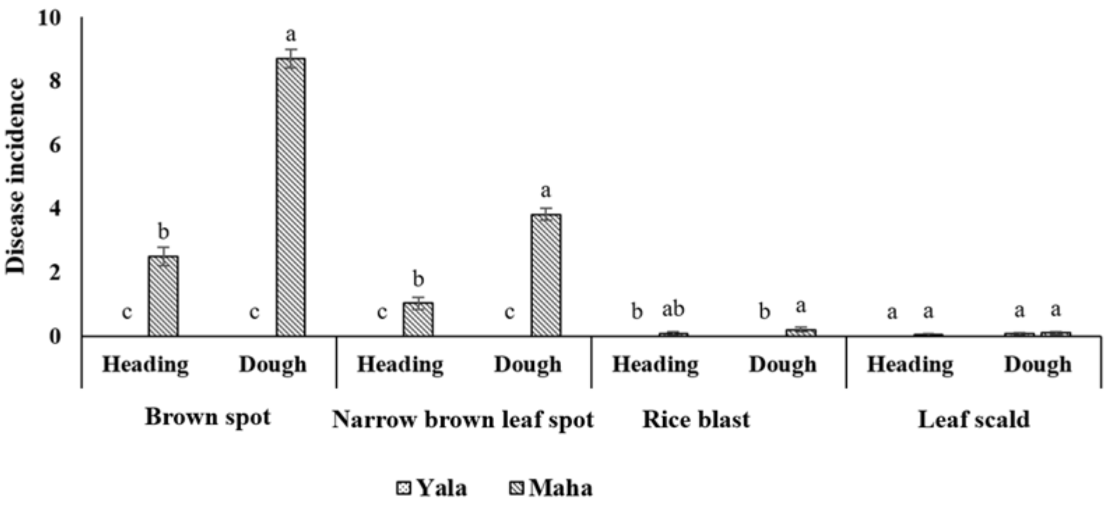


Figure 1. Rice disease incidence at heading and dough stages of rice plant in 2020 *Yala* (dry) and 2020/2021 *Maha* (wet) seasons. Means values followed by same letter in each growth stages in each disease are not significantly different at P<0.05 (LSD).

2006) and germination of fungal spores is favoured in high moisture (Sathischandra et al., 2014). Dry environments have a negative impact on the incidence and progress of fungal diseases whereas wet conditions favour pathogen development (Jain et al., 2019).

*Disease Incidence At Heading and Dough Stages*

Heading and dough stages are the most critical stages of rice crop as the panicles emerge after the booting stage with transitional changes of the reproductive stage (heading) and to grain filling afterward. Detrimental factors may limit the yield formation during reproductive and ripening phases (Figure 2). The development of fungal diseases is common under high humid conditions, relatively long dew periods and cool nights, which are typical in wet season. Furthermore, RB was a significant disease in the early days, thus most of the late improved varieties were bred to be resistant (Groth et al., 1991). The rice variety "Bg300" is moderately resistant to RB (RRDI), therefore it had a low incidence and less economic loss due to RB.

The BS disease incidence was increased around four folds at the dough stage, compared to heading in wet season (Figure 2). Brown spot is the most prevalent disease during the maturity stages of rice (Groth et al., 1991), however, incidences were not severe during

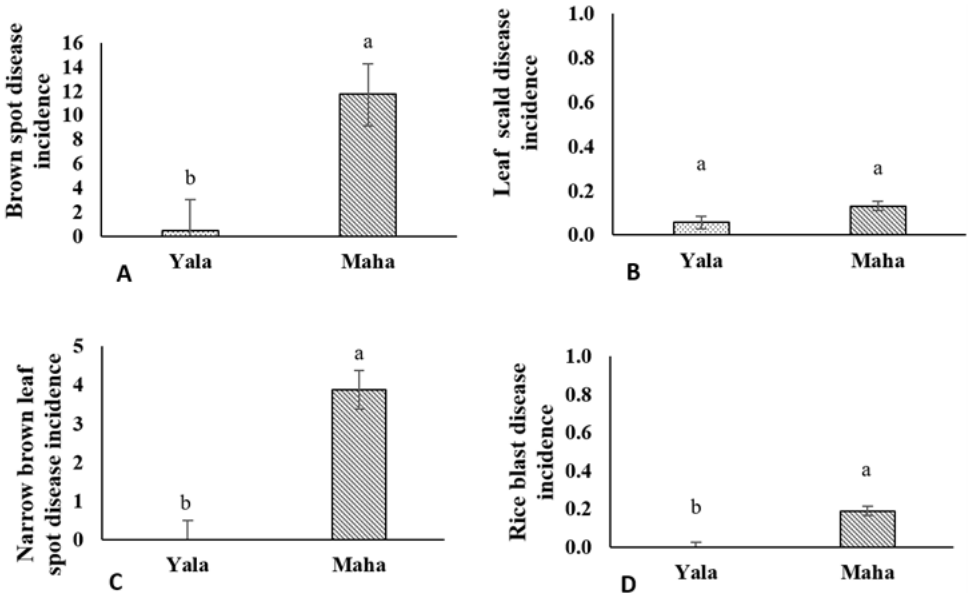


Figure 2. Effect of seasons on the disease incidence of (a) brown spot, (b) leaf scald, (c) narrow brown leaf spot and (d) rice blast.

and that can reduce the final yield substantially. Correct monitoring of crops for diseases during those two stages is helpful in mitigating possible economic losses (Moldenhauer and Slaton, 2001).

At heading, the disease incidences of BS and NBLS were significantly higher in the wet season. The disease incidences of RB and LS were similar and substantially low in both dry and wet seasons (Figure 2). An increasing trend of BS and NBLS were observed and peaked at the reproductive phase, while same trend is well documented (IRRI, 2002). Rice blast starts appearing between seedling and late tillering stages and LS occurs on maturing leaves at the latter stages (Groth et al., 1991).

At the dough stage, the disease incidences BS and NBLS were substantial, but RB was low. Yet, disease incidences were greater than those in the dry season the life cycle of the crop in dry season. The disease incidence of NBLS at the dough stage was also more than three folds higher than heading stage in the wet season, whereas incidences during dry season were negligible. Similarly, incidence of RB was also low in both seasons, while it remained below one (Figure 2).

*Disease Incidences in 2020/2021 Wet Season*

The lower of disease occurrences in the dry season resulted in them being excluded from further analysis. The interaction between sampling time and input system was significant for BS ($P<0.0001$) and NBLS ($P<0.0001$), while for RB ($P=0.4$) and LS ($P=0.97$), it was not significant. The incidence of LS ($P<0.0001$) was significant only due to the impact of input system. The incidence of RB was only affected by the time ($P=0.04$). Diseases like BS, NBLS and LS were dependent on crop rotation and nitrogen fertilizer

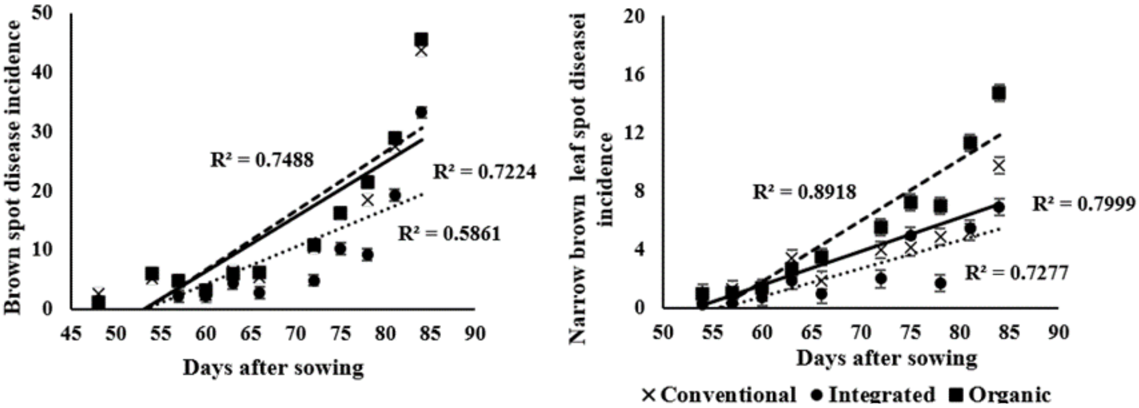


Figure 3. Brown spot (A) and narrow brown leaf spot (B) disease incidence in rice on different cultural systems.

management, while RB incidence was more related to weather conditions during cropping season (Groth et al., 1991).

The disease incidences of BS and NBLS were increasing with crop growth and development irrespective of input systems (Figure 3). The disease incidences of BS in organic and conventional input systems were higher and increased at a higher rate than the integrated system (Figure 3). The incidence of BS showed higher infestation under insufficient nitrogen conditions in organic input system; however, past studies suggested that BS can be managed by proper fertilization (Groth et al., 1991).

The disease incidence of NBLS was higher in conventional input system than the integrated nutrient management system, but lower than that of the organic input system. At 57 DAS when rice crops were at the panicle initiation stage, and 63 DAS at the heading stage, the incidence of NBLS disease in conventional input system was significantly higher than that of both integrated and organic input systems.

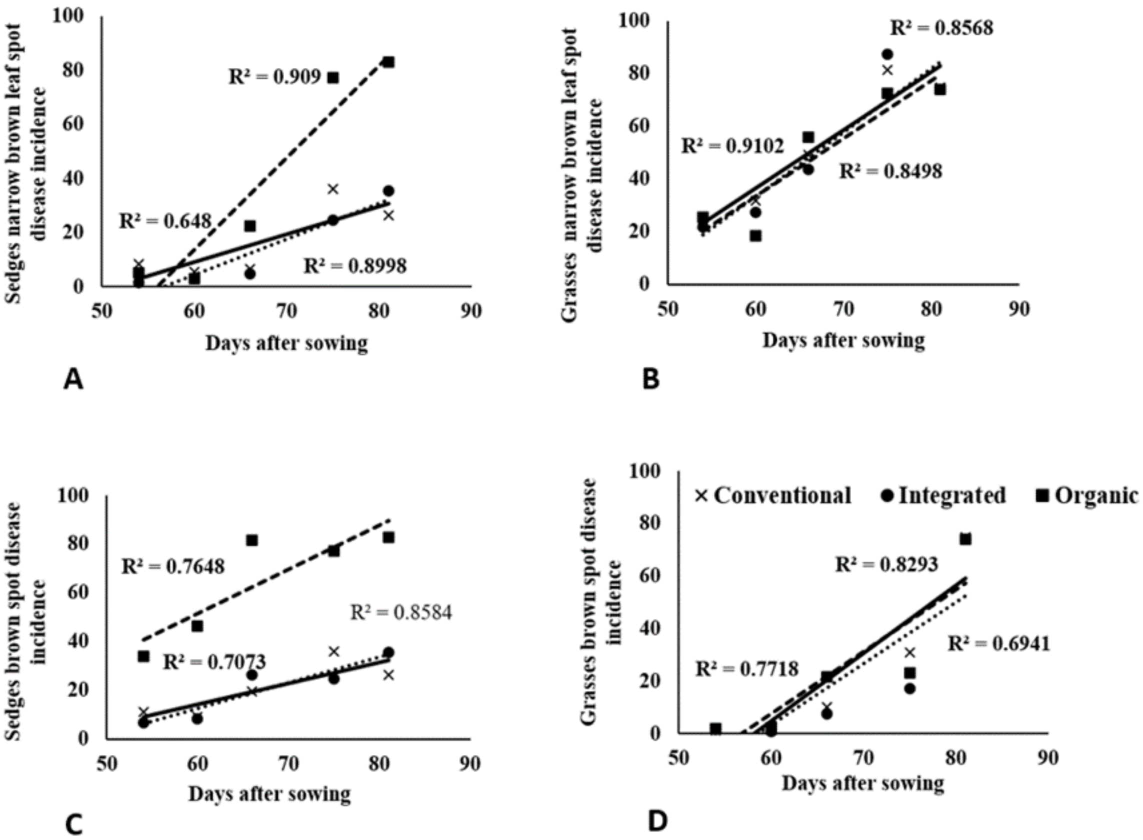


Figure 4. Incidences of narrow brown leaf spot (A) and brown leaf spot (C) on sedges; narrow brown leaf spot (B) and brown leaf spot (D) on grasses in different cultural systems.

However, at the milking stage (75 DAS) and at the soft dough stage (81 DAS), the disease incidence of NBLS in integrated input system was significantly higher than that of the conventional input system and lower than that of organic system (Figure 4).

The disease incidence of RB also recorded an increasing trend. According to Growth et al. (1991), rice blast disease incidence was affected only by the seasonal variations, while no impact from input systems and fertilizer applications were reported.

The LS disease incidence was significantly higher in conventional input system with 0.39 disease incidence than that of integrated and organic input systems, which reported disease incidence less than 0.01. This difference might be associated with the dependence of incidence of LS on nitrogen fertilization. The development of LS disease increases with high rates of nitrogen application and split applications have higher effects than single dosages (Singh and Gupta, 1983).

*Disease Incidence on Weeds*

Weeds act as alternative host plants by harbouring fungal pathogens and transmitting them to the crops via wind and insects when conditions are favourable (Seneviratne and Jeyanandarajah, 2004). Sedges (*Cyperus* spp.) and grasses are commonly reported weed types in the field experiment as alternative host plants for rice diseases. Grasses and sedges are commonly affected by leaf spot diseases like BS and NBLS. Brown spot disease affects grasses comparatively higher than the sedges (Adur-Okello et al., 2020).

The two-way interaction of time and treatment on disease incidence of BS on grasses (P=0.002) and sedges (P=0.02) was significant. However, the same two-way interaction NBLS disease incident was significant only for sedges (P<0.0001). The disease incidence of NBLS on grasses was only significant with the sampling time (P<0.0001).

The disease incidence of BS on sedges and grasses showed an increasing trend with time in all three input systems (Figure 4). The BS disease incidence was higher in the organic input system compared to reduced and conventional systems (Figure 4). The incidences of BS disease on grasses were lower than that on sedges.

The disease incidence of NBLS on sedges and grasses showed an increasing trend with time in all three input systems (Figure 4). The disease incidence on sedges was higher in the organic input system compared to reduced and conventional systems (Figure 4). The incidences of NBLS on grasses were higher at the beginning than that on sedges, and later the incidents were similar.

Grasses and broadleaves were effectively controlled in all systems, but the population of sedges were not effectively controlled. Further, even after herbicide application, sedges were emerged, thus the incidences of sedges were higher proportionate to their presence within systems.

The incidence of BS disease on the rice crop, grasses and sedges showed an increasing trend in organic, integrated, and conventional input systems with time. However, the sedges showed a dissimilar

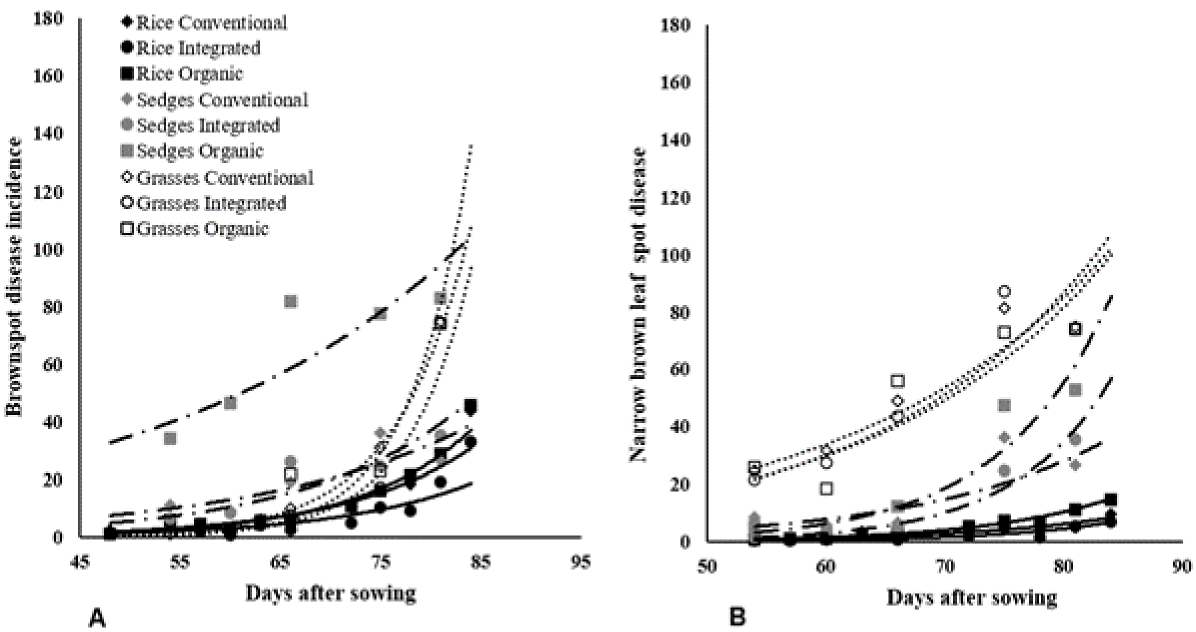


Figure 5. Brown spot disease (A) and narrow brown leaf spot (B) incidence on rice, sedges, and grasses.

trend, as rice and grass weeds belong to the family Poaceae and while sedge weeds belong to the family Cyperaceae (Perera and Dahanayake, 2015). The disease incidence of BS on sedges was substantially higher than that of both rice and grasses from the beginning (Figure 9). This can be due to higher presence of sedges in the systems. Even under low fertilizer systems it can be cause due to seed bank of sedges of previous seasons, slow growth at early days so hand weeding might not have effective, manual weeding, by pulling the entire plant may not have been uprooted.

At the heading stage (65 DAS) disease incidences appearance started increase on rice and grasses, however, the rate was higher on grasses. Grass weeds act as alternative host plants of BS disease.
The incidence of NBLS disease on rice showed an increasing trend that is similar to BS, while NBLS progression on grasses and sedges were different (Figure 5). At 65 DAS (the heading stage), the disease incidences were increased at 65 DAS, while recorded a higher increasing rate than rice and sedges at the beginning. The incidence of rice was the lowest (Figure 5). The disease incidence of NBLS on rice in the organic system was the highest and incidence in the integrated system was the lowest while a similar trend was observed on grasses and sedges too (Figure 5).

*Correlation Between Disease Incidence and Leaf Nitrogen Status*

The disease incidence of BS and NBLS were negatively and significantly correlated to SPAD value and extractable chlorophyll content, while no correlation was recorded to N content (Table 3). Disease incidence of RB was negatively correlated to SPAD value only. The correlations of disease incidence of LS were positive and significant (P<0.05) to all three factors (Table 3). Foliar leaf spot diseases like BS and NBLS affect the foliar nitrogen status of rice leaves (Julião et al., 2020). With seasonal differences, RB disease incidents are not prominent, thus correlations were weak, while no impact of input systems and fertilizer applications were recorded (Groth et al., 1991).

The negative correlations of BS and NBLS were due to the changes in the number of lesions on rice leaf,

Table 3. Associated probability values and the correlation between the disease incidences of brown spot, narrow brown leaf spot, rice blast, leaf scald and nitrogen status of rice in 2020/2021 *Maha* (wet) season.

| Disease incidence | Relative leaf chlorophyll content (SPAD value) | Extractable chlorophyll | Leaf N |
|---|---|---|---|
| Brown spot | -0.60 ** | -0.51 ** | -0.11 ns |
| Narrow brown leaf spot | -0.70 ** | -0.57 ** | 0.004 ns |
| Rice blast | -0.34 ** | -0.23 ns | no |
| Leaf scald | 0.41 ** | 0.37 ** | 0.70 ** |

** significant at P<0.05; ns=not significant; no= not observed.

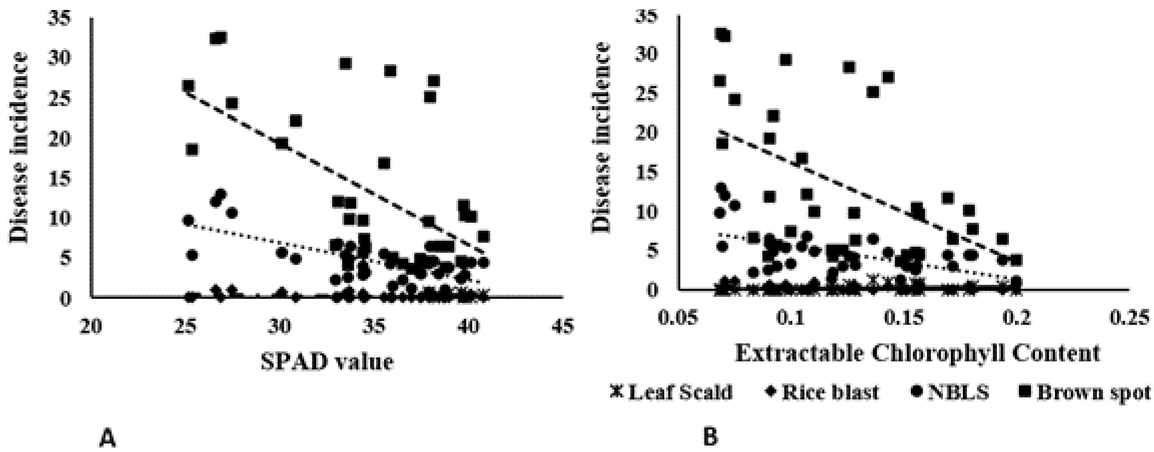


Figure 6. Correlation between diseases incidence and rice leaf SPAD values (A), and between disease incidence and extractable chlorophyll content (B).


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the nitrogen content in leaf tissues decrease. The inverse relationship of BS and NBLS were possible (Carvalho et al., 2010), yet an increase in nitrogen status of crop, in this study is indicated by the SPAD values, generally increases the disease incidence of rice LS (Babu, 2016).

The leaf chlorophyll content indicates the nitrogen status of the rice crop indirectly (Marschner, 1995). The disease incidence of rice blast did not significantly correlate with the chlorophyll content (Figure 6 B).

## Conclusions

Brown spot (*Cochliobolus miyabeanus*) and narrow brown leaf spot (*Cercospora janseana*) diseases were the most recorded fungal diseases during the study. The disease incidence of brown spot and narrow brown leaf spot diseases developed progressively with the development of the crop and peaked around 55 – 65 DAS irrespective of the input system. The highest disease incidences were recorded in the organic input system, while the integrated system recorded the lowest. Nutrient deficiency and imbalance increased the crop's vulnerability to biological stresses such as diseases when microclimate is much more favourable for fungal disease development. Rice nitrogen status affected the incidence of brown spot, narrow brown leaf spot and leaf scald. Brown spot and narrow brown leaf spot disease incidences on weeds were higher in the organic input system. The incidences of brown spot and narrow brown leaf spot diseases on sedges were the highest. The disease incidences were higher on weeds; thus, these diseases can prevail even during the fallow by continuing the life cycle. Hence, judicious management of nitrogen via alternative sources can suppress the disease susceptibility, while without toppling the production expectations. Therefore, an integrated nutrient management system with mineral and alternative sources minimizes the disease incidence and weeds management reduced the persistence of fungal rice diseases in a particular system.

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