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Effect of Nitrogen fertilizer, weed control and seed rate on incidence and severity of narrow brown leaf spot in rice cultivation under the dry zone of Sri Lanka

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Abstract Narrow Brown Leaf Spot (NBLS) caused by *Cercospora janseana* is a common disease of rice causing severe yield loss. In order to identify the factors favourable for disease development under field conditions, a study was carried out during the 2017/2018 *Maha* and 2018 *Yala* seasons. Effects of mineral N fertilizer, weed control, and seed rate on the incidence and severity of NBLS disease were determined. Two levels of mineral N, i.e., Department of Agriculture, Sri Lanka (DOF) recommended level and half of the DOF level were used with weedy and weed-free conditions, under four different seed rates, 100 kg/ha, 125 kg/ha, 150 kg/ha and 175 kg/ha. NBLS incidence was significantly higher ($p<0.0001$) in the *Maha* season compared to the *Yala* season. In the *Maha* season, mineral N fertilizer by weed interaction was significant on disease severity. In the *Yala* season, the disease incidence was significantly ($p<0.05$) higher in weedy conditions and fertilizer, weed and seed rate interaction and fertilizer and seed rate interaction were significantly ($p<0.05$) higher. The lowest disease severity was recorded in both seasons with the 100kg/ha seed rate, 100% fertilizer and weed-free conditions. When the weedy conditions prevailed in the field in *Maha* season, a disease severity scale value of 2 was observed at the highest frequency. Although only the weed condition affects NBLS incidence, it was found that the seed rate, fertilizer and, weed condition interaction was critical to control the severity of NBLS in paddy cultivation.

Keywords: *Cercospora janseana*, N fertilizer, paddy cultivation, *Yala*/*Maha* seasons

1 Introduction

Rice (*Oryza sativa* L.) is the staple food and the most important grain cultivated in Sri Lanka. The total land extent under rice in the country is 792,000 ha and the annual production stands at 2,383,000 MT (Economic and Social Statistics of Sri Lanka, Central Bank of Sri Lanka, 2018). The rice is cultivated in two seasons of the year, i.e., *Maha* and *Yala*, determined by the annual monsoonal rainfall pattern. With the increasing population, there is a demand for increased production of rice. However, it can only be fulfilled through overcoming abiotic and biotic stress conditions (Weerakoon *et al.* 2018). Common biotic stresses encountered in rice cultivation include diseases, insect pests, and weeds. Among these, pathogens causing diseases are major constraints in paddy production that cause considerable yield losses. The fungal disease, Narrow Brown Leaf Spot (NBLS) causes a considerable reduction in the yield of rice cultivation in Sri Lanka (Department of Agriculture, Sri Lanka, 2019). Narrow Brown Leaf Spot caused by the fungus *Cercospora janseana* infects leaves, sheaths, and panicles of the rice plant. The occurrence of NBLS has been recorded in many rice growing areas in the world including the tropics and subtropics of Asia, Australia, Africa and America (Simanjuntak *et al.* 2020). The symptoms appear in the mature stages of the crop reducing the market value of the grains. Light to dark brown linear lesions that are parallel to the veins occur in infected leaves and upper leaf sheaths, enlarging to make large necrotic areas in more susceptible varieties (IRRI 2019).

The low external input and organic crop production are gaining momentum both globally and nationally due to verified negative effects of high input conventional crop production systems on human health and the environment (Reganold and Wachter 2016). The transition towards more sustainable alternative crop production systems is required to cut down external inputs and substitute them with more ecological and biological approaches to manage biotic and abiotic stresses. Ecologically based weed management and integrated soil nutrient management with fewer herbicides and fertilizers have become important in this scenario. Weeds account for 9.5% of annual rice yield losses globally (Alam 2003, Rabbani *et al.* 2011, Hakim *et al.* 2013) and 30–40% in Sri Lanka (Abeysekera 2001, Hakim *et al.* 2013). These unwanted plants can significantly influence disease incidence by acting as pests, serving as an alternative host for pests, pathogens, and vectors (Wisler 2009). The crop has to compete with the weeds for resources subsequently weakening and increasing the probability of pathogen infection (Schreiber *et al.* 2018). It is recommended to remove weeds in the field in order to remove alternative hosts of the fungus, *Cercospora janseana* since the presence of weeds in the field has a direct impact on the NBLS. Furthermore, increasing crop density by increasing seeding rates has been identified as one of the major ecological strategies to out-compete weeds in cereals as well as to increase resource use efficiency. The competitiveness of the crop can be increased by employing a high seed rate (Chauhan 2012, Ahmed *et al.* 2014) to facilitate a quick canopy closure and decreased weed growth (Guillermo 2009, Ahmed *et al.* 2014). However, such ecological changes in the cropping systems can alter other biological and ecological processes due to changes in the microclimate, particularly by negatively influencing the disease development in the crop.

Moving from conventional to alternative production systems can cause a reduction in plant-available N at least during the transition periods. The low availability of N can also influence disease dynamics in crops. Plant growth at high N availability may result in increased plant susceptibility to pathogens due to increased foliar N concentrations (Mitchell *et al.* 2003, Veresoglou *et al.* 2013). However, Panique *et al.* (1997) stated that disease resistance of plants is occasionally reduced due to fertilization, but disease tolerance is increased due to the plant growth stimulating effect caused by nutrient availability (Veresoglou *et al.* 2013).

We hypothesized that the agronomy of rice crops; especially the N status of the crop, and weeds may have an impact on the occurrence of NBLS disease. Therefore, this study was conducted to compare the seasonal variation of incidence and severity of the disease in the *Maha* and *Yala* seasons and to assess the effect of mineral N fertilizer dosage, control of weeds, and seed rates on disease incidence and severity of NBLS.

2 Material and Methods

The experiment was carried out in 2017/2018 *Maha* (2017 November – 2018 March) and 2018 *Yala* (April – August) seasons at the research field of the Faculty of Agriculture, Rajarata University of Sri Lanka (RUSL), Puliyankulama, Anuradhapura. A field trial was established with three treatments; two mineral N fertilizer dosages i.e., full (100%) Department of Agriculture (DOA) recommendation and a half (50%) of DOA recommendation (Table 1) in the presence of weeds, i.e., weedy and weed-free, and at four seed rates, i.e., 100 kg/ha, 125 kg/ha (control),150 kg/ha and 175 kg/ha. The treatments were laid out on a factorial, split-split plot design with four replicates. The size of the plot was 2m x 2m. The N fertilizer rate was the main plot factor and the weed population was the sub-plot factor, while the seeding rate was the sub-sub plot factor. Main plots and subplots were separated with bunds to escape the lateral movement of fertilizers. To avoid the movement of air-borne pathogens, 1.5m height white colour polyethylene sheets were used to cover each plot.

2.1 Crop establishment and management

Pre-germinated rice of variety Bg 300 was direct seeded into puddled soil following the DOA recommendations, in 64 plots established to maintain the treatments during the experiment. Fertilizer application was carried out as split applications according to DOA recommendations.

Weeds were controlled in the weed-free plots by the application of pretilachlor + safener 300 EC according to the recommended rate of 1.6 L/ha, three days after establishment. Bispyribac sodium was then applied at a recommended rate of 225 g/ha 14 days after sowing. Hand weeding was used to control excess weeds in weed-free maintained plots.

Table 1. Treatments and their respective nutrient contents.

Treatment	Mineral nutrient (kg/ha)		
	N (Urea - 46%)	P (P_2O_5 - 16%)	K (K_2O - 60%)
Full (100%) DOA Recommendation	103.5	3.9	30
Half (50%) DOA Recommendation	51.75	1.95	15

DOA: Department of Agriculture, Sri Lanka

2.2 Data collection and statistical analysis

The incidence and severity of NBLS were recorded from the 50 days after sowing (DAS) with four days sampling intervals. Four random quadrate (50cm x 50cm) samples were selected from each plot as described by Lin *et al.* (1979). The number of infected plants in each quadrate sample was counted, and the disease incidence was calculated using the following formula.

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

The severity of the NBLS was assessed by counting the number of infected leaves on four randomly selected plants inside the quadrate (severity 1). Selected plants were tagged with plot number and treatment. The number of spots on the flag leaf of earlier tagged plants was counted. The severity of the disease in the rice plant was estimated using the counted values according to the predetermined scale in Table 2 (severity 2).

Table 2. Severity scale is based on the number of spots on the flag and its description.

Spot numbers/flag leaf	Severity Scale	Description
0-30	1	Less number of spots with individual occurrence. The diameter remains small.
31-60	2	Medium number of spots with individual occurrence. The diameter remains small and medium.
61-90	3	High number of spots with individual and combined patches. The diameter remains small to medium size.
> 90	4	Very large number of spots with individual and combined patches. The diameter remains small to large.

Disease incidence and severity 1 data were tested for normality and heteroscedasticity and the data were transformed to square roots. Non-linear regression analysis was performed to assess disease incidences and severity 1 in both 2017/2018 *Maha* and 2018 *Yala* seasons to identify the pattern of the disease incidence and severity 1. And the effects of each experimental factor in each season were statistically interpreted using SAS statistical software. All treatments and the seasons were considered as fixed factors and the replicate (block) and interactions with sub-plot factors were considered as random factors. All means were separated using Tukey's LSD at p=0.05. To understand the severity of the disease, the severity scale values identified for each block were averaged under each treatment combination.

3 Results and Discussion

Symptoms of NBLS were observed in both the 2017/2018 *Maha* and 2018 *Yala* seasons. The disease incidence and severity were compared with the season and the three crop management factors. The cumulative effect of all treatments for the disease incidence was significantly higher ($p<0.05$) in the 2017/2018 *Maha* season compared to that of the 2018 *Yala* season. *Maha* season recorded an 18% greater disease incidence than that of *Yala* season (Figure 1).

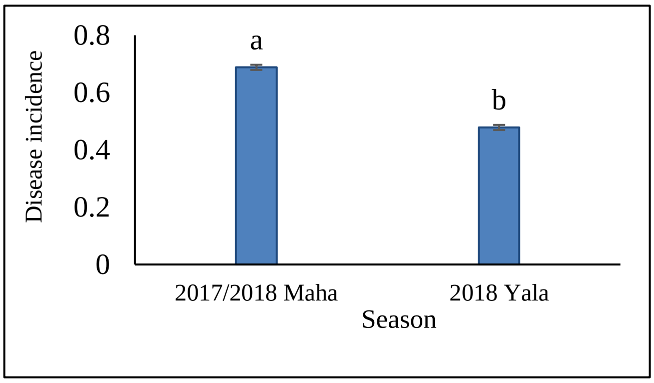

Fig. 1: Cumulative effect of treatment on NBLS disease incidence in 2017/2018 *Maha* and 2018 *Yala* seasons

The observed difference between the incidences of the disease during the two seasons could be associated with the changes in optimal environmental conditions (elevated temperature, humidity and leaf wetness (Pool and McKay 1916, Shane and Teng 1983) or disease development. In the *Maha* season, the development of pathogen may be facilitated by comparatively higher wet conditions due to higher rainfall (data not shown) experienced than that of *Yala* season. It is the most observed trend in the pattern of disease transmission (García-Guzmán and Dirzo 2004), and present results provide evidence parallel to the universal pattern.

The disease incidence in the *Maha* season progressively increased during the period of data collection and reached the maximum by the final sampling day (Figure 2a).

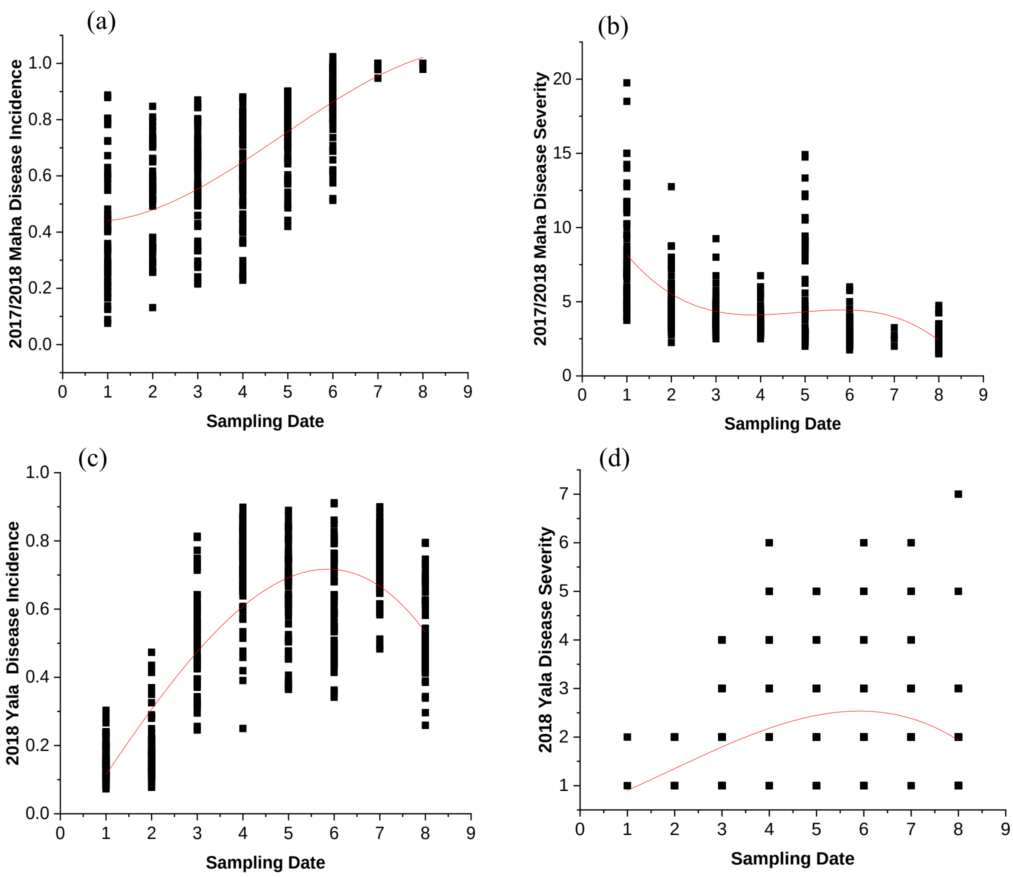

Fig. 2. The cumulative effect of all treatments for NBLS disease.
(a) Disease incidence in 2017/2018 *Maha* season, (b) Disease severity 1 (number of infected leaves per plant) in 2017/2018 *Maha* season, (c) Disease incidence in 2018 *Yala* season and, (d) Disease severity 1 (number of infected leaves per plant) in 2018 *Yala* season.

The favourable microclimate and nutrient uptake during the growing season may be conducive for the growth and spread of the disease during the vegetative and reproductive growing seasons without any hindrance (Krupinsky *et al.* 2002). Although disease incidence gradually increased, disease severity 1 gradually decreased for the first four sampling days (62 DAS), which is also the end of the vegetative growth stage in the *Maha* season (Figure 2b). During that period, plants developed, and the leaf number increased. In proportion to the increasing leaf number, there was no increase in infected leaf number. From the fourth sampling date (62 DAS) to the sixth sampling date (72 DAS), there was a minor increasing trend of disease severity 1 and thereafter a gradual decrease (Figure 2b). From the fourth sampling day to the sixth sampling date, i.e., from the later vegetative period to the initial reproductive stage, leaf growth is not shown while leaf senescence is accelerated. After the sixth sampling date, plants reached the end of the reproductive growth and it might have caused senescence of leaves. It may be the major reason for the decrease in the infected leaves per plant (Vergara 1991).

In the *Yala* season, the disease incidence increased up to the sixth sampling date, thereafter, decreased towards the end of the season (Figure 2c). The incidence of the disease in the *Yala* season increased from a lower level in the vegetative stage of the crop to reach a peak at the initial stage of reproductive growth. The incidence remained the same or decreased during the latter part of the reproductive growth (Figure 2c). Similar results were observed by Long *et al.* (2000) in a study on the incidence of blast disease of rice under different N levels. This pattern of disease progression may be attributed to an aspect of the development of resistance to the pathogen in the plant with maturity. At the end of the vegetative period, the plants become more resistant to certain pathogen due to the physiological maturity (Bastiaans 1993), which alter the chemical composition of cells and permeability of cell walls. The disease incidences were not increased beyond this point as the death of the diseased leaves occur with the formation of new healthy leaves (Long *et al.* 2000).

The present study also observed that the severity of the disease decreases with the growth of the plant. Disease severity 1 in the *Yala* season also showed the same trend of disease incidence and the sixth sampling date reported higher disease severity (Figure 2d). Disease incidence in the *Yala* season and disease severity 1 in both *Maha* and *Yala* was reported maximum values on the sixth sampling date. Thus, the effects of experimental factors on the incidence of the disease and severity were assessed on the sixth sampling date (Figure 2c).

Fertilizer, weed, seed rate and their interactions during the 2017/2018 *Maha* season did not have a significant ($p<0.05$) impact on disease incidence. It was reported that the severity 1 of the disease during the 2017/2018 *Maha* season was significantly different ($p<0.05$) with fertilizer and weed interactions. However, during the 2018 *Yala* season, there was a significant difference ($p<0.05$) in the incidence of diseases with weedy and weed-free conditions. Fertilizer, weed and seed rate interaction significantly ($p<0.05$) influence the disease severity 1 in the *Yala* season. Similar to the incidence of the 2018 *Yala* disease, the severity 1 of the disease varied significantly with weedy and non-weedy conditions (Table 3).

Table 3: Associated probability values for the effect of nitrogen fertilizer, weed control and seed rate on the disease incidence and disease severity 1 in 2017/2018 *Maha* season and 2018 *Yala* season.

Treatment	Disease Incidence		Disease Severity 1	
	2017/2018 *Maha* Season	2018 *Yala* Season	2017/2018 *Maha* Season	2018 *Yala* Season
Fertilizer (F)	0.33	0.09	0.77	0.86
Weed (W)	0.78	<0.01	0.36	0.04
Seed Rate (SR)	0.70	0.89	0.92	0.57
F*W	0.95	0.43	0.03	0.07
F*SR	0.92	0.48	0.70	0.1
W*SR	0.90	0.21	0.57	0.06
F*W*SR	0.97	0.58	0.72	0.04

Weed management and mineral N fertilizer dosage interaction using ANOVA mixed model was significant ($p<0.05$) for the disease severity 1 in *Maha* season while all factors did not significantly ($p>0.05$) affect disease incidence in *Maha* season. A higher disease severity (3.3 leaves/ plant) was observed in plants grown in weedy conditions and supplied with the full recommendation of mineral N fertilizer than that of the treatment with half the dosage of N fertilizer in weedy condition (Figure 3).

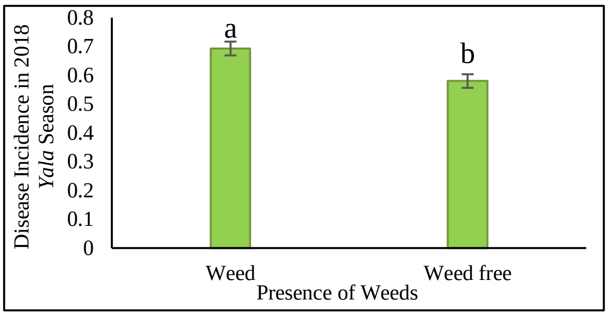

Fig. 3. NBLS disease severity in 2017/2018 *Maha* season with the rate of Nitrogen fertilizer and control of weeds

Significantly lower disease severity (2.06 leaves/ plant) was reported with the weed-free condition with the full recommendation of mineral N fertilizer. Effects of other factors and interactions were insignificant ($p>0.05$) (Table 3). Generally, the presence of high N in plants with a high N supply can increase the susceptibility to diseases (Makheti Mutebi *et al.* 2021). The invasion and growth of fungi can be facilitated by the weakening of the mechanical resistance induced by cell wall materials due to high N applications (Talukder *et al.* 2005). Since some weeds act as a host for diseases, weedy conditions may negatively affect the crop (Table 4). More sedges were reported than grasses, broad leaves and aquatic weeds in both seasons. As sedges, *Cyperus rotundus* (Kaladuru) (more abundance), *Echinochloa crus-galli* (Bajiri), and *Fimbristylis dichotoma* (Kudametta) (low abundance) were reported (data not shown) with this experiment. Biswas (2006), also reported, sedges are the host plant for the narrow brown leaf spot. Complete control of weeds under the full fertilizer dose can be used as a remedy to control the severity of the disease in the *Maha* season (Groth *et al.* 1991).

Table 4: Mean disease incidence, severity 1 and severity scale values of treatment combinations in 2017/2018 *Maha* season and 2018 *Yala* season on sixth sampling date of rice crop.

Treatments			Disease incidence		Severity 1		Severity Scale Value	
Seed Rate	N fertilizer Dosage as % DOA	Presence of Weed	2017/2018 *Maha* Season	2018 *Yala* Season	2017/2018 *Maha* Season	2018 *Yala* Season	2017/2018 *Maha* Season	2018 *Yala* Season
100 kg/ha	100%	Weedy	0.81^{a}	0.63^{abcd}	3.73^{ab}	2.83^{a}	2	1
		Weed Free	0.86^{a}	0.51^{d}	2.38^{b}	1.39^{b}	1	1
	50%	Weedy	0.82^{a}	0.78^{a}	2.94^{ab}	2.87^{a}	2	1
		Weed Free	0.84^{a}	0.62^{abcd}	2.87^{ab}	1.90^{ab}	2	1
125 kg/ha	100%	Weedy	0.84^{a}	0.71^{abc}	2.93^{ab}	2.24^{ab}	1	1
		Weed Free	0.85^{a}	0.54^{cd}	2.79^{ab}	1.72^{ab}	2	1
	50%	Weedy	0.88^{a}	0.78^{ab}	3.00^{ab}	2.90^{a}	2	1
		Weed Free	0.85^{a}	0.51^{d}	3.16^{ab}	2.05^{ab}	2	1
150 kg/ha	100%	Weedy	0.87^{a}	0.66^{abcd}	3.21^{ab}	2.69^{ab}	2	1
		Weed Free	0.81^{a}	0.59^{bcd}	2.56^{ab}	2.04^{ab}	1	1
	50%	Weedy	0.91^{a}	0.61^{abcd}	2.81^{ab}	2.40^{ab}	2	1
		Weed Free	0.89^{a}	0.62^{abcd}	3.82^{a}	3.01^{a}	2	1
175 kg/ha	100%	Weedy	0.87^{a}	0.60^{abcd}	3.31^{ab}	2.00^{ab}	2	1
		Weed Free	0.85^{a}	0.63^{abcd}	2.69^{ab}	2.16^{ab}	1	1
	50%	Weedy	0.92^{a}	0.77^{ab}	2.75^{ab}	2.6^{ab}	2	1
		Weed Free	0.90^{a}	0.59^{bcd}	2.77^{ab}	1.8^{ab}	1	1
CV %			7.78	10.56	15.09	22.73	-	-

DOA: Department of Agriculture; Absence of shared superscripts within columns indicate significant difference (ANOVA, $p<0.05$).

Disease incidence was significantly ($p<0.05$) higher when rice was grown under weedy conditions (69%) than weed-free conditions (58%) in the *Yala* season (Figure 4), while the impacts of other factors were constant. Islam *et al.* (2002), observed similar results, where there was a significant ($p<0.05$) effect from weeds to increase diseases in plants. The same study explained the reduction of the disease when the source of the pathogen was reduced in the field through the removal of weeds. Moreover, the presence of weeds increased the relative humidity and decrease the temperature surrounding the crop creating a favourable microenvironment for the pathogen (Sall 1981) making the plants grown among weeds more susceptible to the disease. This might be the reason for the recorded higher disease incidence in the *Yala* season. Also, weed cause to increase disease susceptibility of the crop as a result of competition exerts by weed for the resources such as nutrients water, etc. In this study, it was stated that this factor also contributed to the increase in the disease.

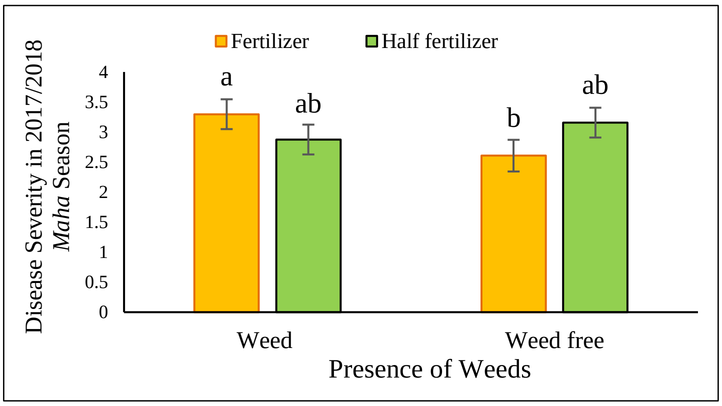

Fig. 4. NBLS disease incidence in 2018 *Yala* season with control of weeds.

During the *Yala* season, the incidence of disease in the plot with the seed rate of 125 kg/ha, 50% fertilizer and weedy were higher than the disease incidence in the weed-free plot (Table 4). Half of the fertilizer application rate reduces the vigour of the plant, thereby increasing the susceptibility of the plant to disease (Huber *et al.* 2012). Disease severity 1 in the *Yala* season with 100 kg/ha rate, 100% fertilizer dosage and weedy condition was reported significantly ($p<0.05$) higher value than disease severity in non-weedy conditions with same seed rate and same nutrient management conditions. The 100kg/ha seed rate, 100% fertilizer and weed-free condition reported the lowest disease severity in both *Yala* and *Maha* seasons (Table 4). It also interprets that weed control is important under all seed rate and fertilizer levels to control the disease development (Groth *et al.* 1991).

In addition to identifying the incidence of the disease and the number of infected leaves per plant, the severity of the disease was determined by calculating the number of spots on a flag leaf with the scale values (Table 4). When the weedy conditions prevailed in the field in the *Maha* season, the disease severity scale value of 2 occurred at the highest frequency. Groth et al. (1991) also reported that weedy conditions caused higher disease severity than weed-free conditions at any seed rate and any fertilizer management practices.

4 Conclusions

According to the findings, the incidence of the NBLS disease was higher during the *Maha* season than that of the *Yala* season. The disease incidence and severity increased with the application of high mineral N fertilizers with the presence of weeds in the *Maha* season, while the disease incidence increased with weedy conditions only in the *Yala* season. Disease severity in the *Yala* season significantly changed with the seed rate, weed and fertilizer dosage interaction. The seed rate had no significant effect on the disease incidence in the two seasons. This study concludes that controlling weed significantly contributes to the reduction of NBLS disease.

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