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RESEARCH ARTICLE

EFFECT OF VARIABLE RATE APPLICATION ON RICE LEAVES BURN AND CHLOROSIS IN SYSTEM OF RICE INTENSIFICATION

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ABSTRACT

Both nitrogen deficiency and over-fertilization result in rice leaf damage and effect on yield production. Insufficient nitrogen supply gives raise to yellow leaves, whereas spraying too high concentrations of fertilizers can be recognized by leaf burn. This study used variable rate application (VRA) of organic foliar fertilization to fertilize the System of Rice Intensification (SRI) cultivation without application of granular fertilizer and used Soil Plant Analysis Development (SPAD) chlorophyll meter as a tool to measure chlorophyll-nitrogen content for fertilizer calculation. Based on a greenhouse experiment the effect of nitrogen regime on rice leaves damage was assessed. The experiment consisted of four nitrogen regimes (50% fixed rate, 100% fixed rate, 150% fixed rate and VRA) arranged in a randomized complete block design (RCBD) with four replications. Experimental result showed that none of the treatments resulted in leaf burn, whereas chlorosis was observed for all the treatments. VRA had the lowest level of chlorosis with low supplied of nitrogen compared to uniform treatments. Application of organic liquid fertilizer in variable rate form and using SPAD chlorophyll meter able to help to diagnose accurately the nitrogen content in the rice leaves for fertilizer application and capable to reduce chlorosis effect on the rice leaves.

KEYWORDS

nutrients deficiency, leaf burn, chlorophyll-nitrogen content, SPAD meter, precision farming.

1. Introduction

Shortage of major nutrients such as nitrogen is a serious issue in rice cultivation since the nutrients deficiency can lead to plant chlorotic condition and limits the rice yields (Chen and Wang, 2014). Nitrogen deficiency on rice plant can be noticeable during vegetative and reproductive growth due to insufficient rates of nitrogen fertilization, and fertilizer loss to environment. In fact, nitrogen deficiency in soil was also caused by other reasons such as loss of nitrogen due to soil erosion, leaching, volatilization, and denitrification (Alam et al., 2015). Since nitrogen is one of the key limiting nutrients in rice cultivation, therefore, knowing the rice nutrients status and finding accurate nitrogen level required by the rice is crucial in enhancing growth development and yield (Gholizadeh et al., 2017).

Leaf yellowing is the most frequently observed nutrient deficiency symptom in rice production including in the System of Rice Intensification (SRI). Yellowing on leaves usually will change to yellow-pale or yellow white and if the deficiency is too severe the leaves will fully turn into yellow-brown colour. While other numerous symptoms are also shown by the rice plant whenever having nitrogen deficiency such as reduction in tiller number, low panicle count, reduction in spikelet number and low grain count (Fageria and Santos, 2015). Commonly, leaf yellowing is the chlorotic condition happened due to low rates of fertilizer supplied to the plant. Chlorosis is as a condition where the rice leaves produce low chlorophyll content and affect the green colour of the leaf therefore related to the leaf nitrogen content in the plant (Shayganya et al., 2011).

Chlorophyll is mainly responsible for effective photosynthesis process which use sunlight to produce foods or substances within the plant cell for plant growth and development. If nitrogen deficiency occurred in plant, it is unable to produce sufficient chlorophyll and caused leaves to become light green-yellow pale and chlorotic at the tip especially for old leaves. While for young leaves, the leaves will become narrow, short, erect and yellowish. Leaf chlorosis is also a sign of inefficient photosynthesis within the rice plant and photosynthesis decreases as the severity of nitrogen deficiency increases (Muhidin et al., 2018).

Usually, nitrogen deficiency symptom is diagnosed by using visual morphological diagnoses in the field but it is tedious and require vast amount of experiences to accurately diagnose the deficiency (Lee and Lee, 2013). Fortunately, a hand-held tool such as Soil Plant Analysis Development (SPAD) chlorophyll meter is created and has the advantage to diagnose accurately the nitrogen deficiency in the plant since SPAD values has a relationship between leaf chlorophyll concentration with nitrogen content (Liu et al., 2015). Currently, SPAD chlorophyll meter widely used in rice cultivation including SRI cultivation for fertilizer management specially to improve nutrient-nitrogen based fertilizer application (Ghosh et al., 2013).

Foliar fertilization is practiced widely in most cropping system as well as maintaining soil fertilizer application within the cultivation due to its advantages to minimize the soil-environment degradation by reducing the amount of granular soil based application through reduction of nutrients leaching, runoff, drainage and ground water contamination (Wang et al.,

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2017). Hence, foliar fertilization capable to increase agronomic performances of plant and increase plant yield compared to the granular method (Saberioon et al., 2013). Most of the time, foliar fertilization is practiced only to supplement micronutrient elements in rice cultivation to correct nutritional deficiency problem of the plant but the rates of the application still in uniform rate (Rabin et al., 2016). However, leaf burn on the plant can be observed after spraying the foliar fertilizer which could indicate high dosage of fertilizer rates used or improper handling during the foliar fertilization process.

Even though there are many researches about foliar fertilization that showed many advantages however there were less information on the leaves burn and chlorosis effect on the rice plant by using only foliar fertilization as solely fertilizer sources to supply macro nutrients in rice cultivation. In fact, there was less information on the application of precision farming principle within the SRI fertilizer management since most of the SRI cultivation relies on the uniform rates of fertilizer application either for organic or inorganic type of fertilizer (Lee et al., 2009). The aim of the study is to understand the actual needs of the nutrients that required by the rice plant before nutrient sources applied to prevent the incident of leaf burn and chlorosis symptoms. Thus, the main objective of the study is to highlight the usage of variable rate application technique by using SPAD chlorophyll meter as a tool to apply precisely the variable rate organic fertilizer in the SRI and to understand the close interaction between the chlorosis-nitrogen deficiency symptom while preventing leaf burn effect on the rice leaves.

2. MATERIAL AND METHODS

2.1 Site and soil

The experiment was conducted at Ladang 2, Universiti Putra Malaysia $(3.0087^{\circ}\ N,\,101.7037^{\circ}\ E)$ from January 1, 2017 until April 30,2017. Rice cultivar MR219 was planted in pots of 40 cm height and 34 cm diameter size filled with sandy clay loam soil in weight of 15 kg. The experimental soil used have pH 6.8, soil organic matter 5.40%, total nitrogen 0.25%, available phosphorus 230.8 kg/ha, available potassium 287.6 kg/ha and CEC 18.3 cmol kg⁻¹ soil.

2.2 Treatment and cultural operations

An organic foliar fertilizer (fish based) was used in this experiment as foliar spraying application with nitrogen, phosphorus, and potassium of 4.8:4:3.5. Foliar spraying was performed at early crop establishment – 15 Days After Transplanting (DAT), mid-tillering (35 DAT), panicle initiation (55 DAT) and flowering (65 DAT). This experiment only took into account of nitrogen content in the leaves for fertilizer amount calculation while phosphorus and potassium ratio were formulated according to supplier recommendation. The experiment consisted four type of treatments; 50% fixed rate, 100% fixed rate, 150% fixed rate and variable rate application (VRA). Each treatment had four replications arranged in a randomized completely block design (RCBD).

Treatment of 50% fixed rate, 100% fixed rate, and 150% fixed were applied based on the supplier recommendation without using SPAD chlorophyll meter measurement. While the VRA treatment was performed based on SPAD chlorophyll meter measurement to measure the rice leaf chlorophyll content which later transferred into nitrogen fertilizer formulation to recommend precise nitrogen amount that actually required by the rice plant. A handheld pump sprayer was used in the experiment to performed foliar spraying on top of the canopy of the rice leaves. The rice seeds were pre-germinated in the SRI nursery tray for 10 days and then transferred into the pot with one seedling planted per pot (Zubairu et al., 2015). The pots were arranged in a line according to each group of treatment with widely space and planting distance between the pots of 34 cm x 34 cm.

SRI planting practices were performed throughout the period of rice cultivation until the harvesting the process, where the water regime was maintained at the moist condition level only and weeding were performed for every planting pots. Water conditions in the pots were monitored every day until the moist condition was maintained and whenever the soil in the pot looked too dry additional water was added. Repeated weeding in the pot was performed every 10 days by using hand and fork weeder to aerate the soil, remove weeds and incorporate them into the soil as exactly as the SRI method practiced in the field. Adequate pest and disease control measures were taken throughout the plants' growth according to SRI recommendation such as application of liquid neem-based bio-pesticide to spray all over the rice plant at every planting stages to reduce pest and disease attack to avoid further variability.

2.3 SPAD measurement

Chlorophyll meter (SPAD-502, Minolta Camera Co., Japan) was used in the experiment for taking the SPAD reading before and after foliar fertilization application; at the early crop establishment (15 & 18 DAT), midtillering (35 & 38 DAT), panicle initiation (55 & 58 DAT) & flowering (65 & 68 DAT). Outermost and fully expanded leaves of rice plant were taken at the tip, midway and base of the leaf and then were averaged (Yuan et al., 2016). Hence, SPAD reading for the rice plant were also measured for every 10 days until 95 DAT before the harvesting process.

2.4 Nitrogen application formula

The nitrogen application formula was modified to determine the amount of nitrogen for VRA foliar fertilization treatment in the experiment to develop threshold chlorophyll values for rice plant in the region of Malaysia (Gholizadeh et al., 2011). At early crop establishment (15 DAT) until mid-tillering (35 DAT), formula (1) was used to determine nitrogen content; N (mg/L) = 0.80 + 0.93*SPAD. Then, at panicle initiation (55 DAT) and flowering (65) stages, formula (2) was used to determine nitrogen content; N (mg/L) = -2.61 + 0.98 *SPAD. Overall formula (3) to determine amounts of foliar fertilization (mL) needed for liquid spraying was; (mL) = [A - (formula (1) or (2) / 1000)] / C, where: A is the threshold level of N in the rice leaves in mg/mL while C is the N amount in percentage of the foliar fertilization used.

At early crop establishment (15 DAT) and mid-tillering (35 DAT)

Nitrogen (mg/L) = 0.80 + 0.93*SPAD (modified after Gholizadeh et al. [16])(1)

At panicle initiation (55 DAT) and flowering (65 DAT)

Nitrogen (mg/L) = -2.61 + 0.98 *SPAD (modified after Gholizadeh et al. [16](2)

Amount of Liquid Fertilizer (mL) = [A - (1 or 2 / 1000)] / C(3)

2.5 Plant sampling

Plant sampling was performed to measure the number of chlorosis and leaf burns on rice leaves were conducted from 15 DAT until 95 DAT for all the treatment. All the sampling was collected and recorded as parameter data to determine the effect of chlorosis and leaf burn on rice leaves between VRA and uniform fertilizer rates.

2.6 Statistical analysis

For statistical analysis of data, Statistical Analysis System Software (SAS 9.1, SAS, USA) was employed. All data collected were subjected to analysis of variance (ANOVA) and mean values between treatment rates were compared using Tukey's honest significant difference (HSD) test at 0.05.

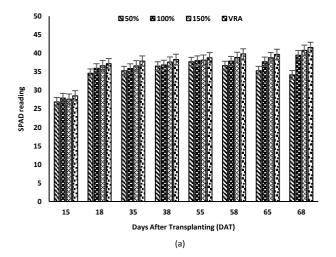
3. RESULTS AND DISCUSSION

3.1 SPAD values

Figure 1 (a) and (b) show the SPAD reading pattern of all the treatments which varied according to different DAT. Figure 1(a) show the SPAD readings of rice plant that had been taken before and after foliar fertilization in order to measure the effectiveness of foliar spraying at different rates to rice plant. Treatments of 100% fixed rate, 150% fixed rate and VRA show increase in SPAD readings from 15 DAT until 68 DAT with less chlorosis deficiency symptom on the rice leaves. However, only 50% fixed rate shows decline in SPAD readings after foliar fertilization between 55 - 58 DAT and 65 - 68 DAT where higher chlorosis count was observed which indicate less amount of chlorophyll content in the rice leaves due to insufficient of nitrogen supply from the foliar fertilization to the plant. Active process for panicle initiation is happened during period of 55 - 68 DAT and require a lot of amount of nutrients especially nitrogen since nitrogen is essential substance of amino acid, nucleic acid, chlorophyll, protein (enzymes) and others for panicle production and multiplication (Foulkes and Murchie, 2011; Hu et al., 2014).

While during flowering period which happened during 65 – 75 DAT, rice plant requires more nutrients of phosphorus and potassium however still required nitrogen for grain production since nitrogen can affects all parameters contributing to yield. While from Figure 1(b), VRA shows the highest SPAD readings pattern throughout the planting period until 95 DAT compared to other treatments. However, treatments of 150%, 100%

fixed rates and VRA show decline in SPAD reading from 75 DAT until 95 DAT after the last cycle of foliar fertilization during 65 DAT. While, treatment of 50% fixed rate shows the lowest SPAD readings pattern and show decline trend in SPAD reading from 55 DAT until 95 DAT which indicate that it is unable to supply sufficient nutrients to rice plant for grain production until grain maturity process. Sufficient nutrients source especially nitrogen was essential to develop sufficient chlorophyll content in the leaf for efficient and effective photosynthesis process and if there was deficiency, reduction of plant photosynthetic rate could happen and cause declining in plant growth and developme thus yield contribution (Amane, 2011).



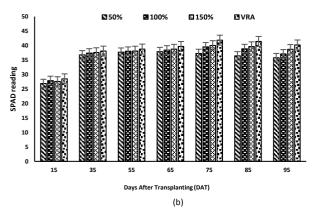


Figure 1: SPAD reading of rice leaf for different fertilizer treatment rates (a) SPAD reading before and after foliar fertilization to the plant at four split time of fertilization (b) SPAD reading of rice leaf recorded on various DAT.

3.2 Chlorosis occurrence on rice leaves

Chlorosis occurrence on the rice leaves was determined through visual observation by detecting the discoloration on the leaves from green to yellow whether on certain leaf regions or complete region of leaf. Commonly, chlorosis occurrence occurred earlier at older leaves compared to new leaves since nitrogen was a mobile type of nutrient deficiency and because of its high mobility, it easily lost to the plant and soil. However, after a few days of nitrogen fertilizer application, the rice leaves change back to green colour due to nitrogen high mobile ability. Reduction of nitrogen content in leaf can be close relationship to leaf chlorosis and a sign of the reduction in photosynthesis process in the plant due to less function of chlorophyll to reflect green light to produce green colouration (Mona et al., 2012). As can be seen in the Table 1, number of chlorosis was observed and counted on every 10 days until 95 DAT before harvesting process. All the treatment rates had chlorosis occurrence on rice leaves in the early stages (15 DAT).

The occurrence of chlorosis on rice leaves keep happening throughout the planting period until 95 DAT without showing any decline in chlorosis count even after foliar fertilization was performed. The highest chlorosis count was observed on rice leaves of 50% fixed rate treatment followed by 100% fixed rate, 150% fixed rates and VRA was the lowest in the chlorosis count as compared to other treatments. The chlorosis occurrence on the rice leaves might indicates the early sign of reduction in plant leaves chlorophyll contents and reduction of chloroplast number in

leaf which was responsible for leaf yellowing due to nitrogen deficiency in the rice plant. The increment in chlorosis count on the rice leaves from 15 DAT until 75 DAT was usually happened because rice requires nitrogen almost throughout the vegetative cycle however more demanding during period of tillering and panicle initiation stages was in critical and need in large amount (Fageria and Santos, 2015).

The accumulation of nitrogen was begun in the leaves during vegetative phase (15 – 35 DAT), then migrates to panicles (55 -65 DAT) and last during the grain maturity (75 – 95 DAT) period. Mostly nitrogen was absorbed by the rice in large quantities for sufficient growth, development, yield and during grain maturity stages, 75% of the nitrogen assimilates in the grain. That could be the reason why during period of 85 – 95 DAT chlorosis occurrence was increase dramatically while SPAD reading of rice leaf was declining for all the treatments since nitrogen was consumed in high amount by the rice plant during this period for the grain maturity process. From the Figure 1 and Table 1, it can be said that uniform application such as 50%,100%, and 150% fixed rate of foliar fertilization unable to provide sufficient nutrients throughout the planting period of rice plant due to high accumulation of chlorosis count.

Meanwhile, rice plant that received foliar fertilization by using VRA method showed less chlorosis count during entire planting period because of accurate nutrients adjustment was performed based on actual needs by the rice plant by using VRA-SPAD chlorophyll meter measurement method. SPAD chlorophyll meter was proven to predict accurately the nitrogen needed by rice plant based on current nutrients availability in the soil and plant requirement for specific application of foliar fertilization amount and concentration rates in SRI cultivation. Therefore, VRA foliar fertilization by using SPAD chlorophyll meter was useful during site – specific fertilizer application especially for foliar fertilization to provide sufficient nutrients to the rice plant for healthy growth with less deficiency occurrence on the rice leaves.

| Table 1: Comparison of chlorosis counts between all the treatments on various DAT (NOS) | | | | | | | | | | | |
|--|-------|-------|-------|--------|-------|--------|--------|--|--|--|--|
| Treatments | 15 | 35 | 55 | 65 | 75 | 85 | 95 | | | | |
| 50% | 1.00a | 2.50a | 3.25a | 4.00a | 5.00a | 8.00a | 9.00a | | | | |
| 100% | 1.00a | 2.25a | 2.75a | 3.25ab | 4.50a | 6.00ab | 8.5ab | | | | |
| 150% | 1.00a | 2.00a | 2.50a | 3.00ab | 4.25a | 5.50b | 7.00ab | | | | |
| VRA | 1.00a | 1.25b | 2.25a | 2.75b | 3.75a | 4.75b | 6.00b | | | | |

*means separation in each column followed by the same letter are not significantly different at p = 0.05

3.3 Effect of weather patterns on chlorosis count

Weather patterns such as relative humidity, solar radiation, sunshine hours, evapotranspiration and max temperature are important factor that play significant role in rice productivity therefore could affect the rice physiology growth during the study (Basak et al., 2010). In the study, weather pattern characteristics such as relative humidity, solar radiation, sunshine hours, monthly rain precipitation, evapotranspiration and max temperature can be seen in Table 2. Weather patterns were varied throughout the planting period according to the rice growth stages and since the experiment was conducted in greenhouse-controlled experiment so weather characteristics such as rainfall, max and min temperature were suitable and response accordingly as mentioned (Ablar et al., 2017). Lower solar radiation and sunshine hours during the planting period could had reduced the photosynthesis process of the plant therefore less amount of chlorophyll content produce which can lead to yellowing of the leaf (Sun et al., 2012). Hence, due to heavy clouding that caused shading might had lower the solar radiation and sunshine hours during the planting period (Chen et al., 2019).

While for relative humidity characteristic, high relative humidity was found to be anticipated. High relative humidity can influence the evapotranspiration rate which high evapotranspiration rate may influence the plant physiological process (Irmak, 2017). High relative humidity can reduce the evapotranspiration rate which later can turn liquid fertilizer droplet into more atmospheric vapour. This can cause less absorption of liquid droplets into the leaves and cause the plant not sufficiently received enough nutrients from fertilizer spraying. So, this indirectly could cause more chlorosis effect on the leaves which also indicate signed of nutrients deficiency. Therefore, field weather pattern is important for consideration in planning the application of liquid fertilizer to ensure the optimization of liquid spraying effect to the plant in order to minimize the chlorosis symptoms on the leaf which later can enhance the overall rice plant health and produce better yield.

| Table 2: Pattern of meteorological data at Ladang 2, University Putra Malaysia from January until April 2017 (retrieved from CLIMWAT, FAO). | | | | | | | | | | |
|---|-------------------------------|--------------|--------------------------------|---------------------------|-----------------------|----------------------|----------------------|--|--|--|
| Month | Rain precipitation (mm/Month) | ETo (mm/day) | Solar radiation (MJ/m²/day) | Sunshine hours (h/day) | Relative humidity (%) | Max temperature (°C) | Min temperature (°C) | | | |
| January | 162.8 | 3.54 | 17.82 | 6.11 | 85.48 | 31.9 | 22.1 | | | |
| February | 144.7 | 3.81 | 19.30 | 6.56 | 88.81 | 32.8 | 22.3 | | | |
| March | 218.4 | 4.06 | 20.00 | 6.73 | 84.38 | 33.1 | 22.8 | | | |
| April | 284.8 | 3.92 | 19.41 | 6.56 | 87.8 | 33.0 | 23.4 | | | |

4. CONCLUSION

The present study shows there was no any incidence of leaf burn on the rice leaves at all fertilization rates. By using SPAD chlorophyll meter measurement, less chlorosis occurrence was observed on the rice leaves compared to treatment that used uniform fertilization without relies on SPAD chlorophyll meter measurement. It can be concluded that rice plant only required specific amount of nitrogen at specific time of its growth stages and precise fertilization can benefit the SRI cultivation to lessen the chlorosis occurrence and to prevent leaf burn from excessive foliar fertilization.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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