EFFECT OF CHITOSAN AND CHEMICAL FERTILIZERS ON THE VEGETATIVE GROWTH PERFORMANCE OF DRYLAND PADDY

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ABSTRACT

This study was conducted to evaluate the effect of chitosan and chemical fertilizers concentrations on the vegetative growth performance of dryland paddy. The chemical fertilizers used were urea, TSP and MOP in a 120-60-60 ratio. This field experiment used factorial completely randomized design (CRD) and took three months. The treatments were: (i) 0 ppm chitosan (T1) (ii) 40 ppm chitosan (T2) (iii) 80 ppm chitosan (T3) (iv) 120 ppm chitosan (T4) (v) 100% NPK (F1) (vi) 75% NPK (F2) (vii) 50% NPK (F3) (viii) 25% NPK (F4). 100% NPK was defined as NPK ratio of 120-60-60. Sixteen different treatment combinations were applied and each treatment combination was replicated four (4) times. Six parameters were recorded in this experiment including plant height, number of tillers, main culm diameter, chlorophyll content at panicle initiation, total fresh weight and total dry matter. Soil was analyzed for pH, N, and available P before and after the experiment. All data were analyzed using ANOVA at 5% level of significance. The ANOVA results for plant height, number of tillers, main culm diameter, chlorophyll content, and total soil nitrogen show no significant difference between the different concentrations of chitosan and fertilizers. The interaction between T4 and F2 resulted in a significantly higher fresh weight (231.5 g) compared to other interactions. It was 88% greater than the interaction between T2 and F3 (122.6 g). The interaction between T4 and F2 resulted in a 64.9% significantly higher dry matter (44.9 g) compared to T1 and F4 (27.2 g). For soil pH, the interaction between T4 and F4 shows the highest value (6.6) and was 8.6% greater than the interaction between T1 and F1 (6.1). The interaction between T4 and F3 resulted in a significantly higher soil available phosphorus (0.52 mg kg^{-1}). It was 92% greater than the interaction between T1 and F2 (0.27 mg kg^{-1}). The results indicate the mild effect of chitosan incorporation for several vegetative growth parameters. However, the positive effects of chitosan to the plants are not negligible and a longer term study is needed to see the real potential of chitosan especially to the yield of dryland paddy.

KESAN KEPEKATAN KITOSAN DAN BAJA KIMIA TERHADAP PERTUMBUHAN VEGETATIF PADI HUMA

ABSTRAK

Kajian ini telah dijalankan untuk mengenal pasti kesan kepekatan kitosan dan baja kimia terhadap pertumbuhan vegetatif padi huma. Baja kimia yang telah digunakan adalah urea, TSP dan MOP dalam nisbah 120-60-60. Kajian ini dijalankan dengan menggunakan rekabentuk rawak lengkap faktorial (CRD) dan mengambil masa selama tiga bulan untuk diselesaikan. Rawatan yang terlibat adalah seperti berikut: (i) 0 ppm kitosan (T1) (ii) 40 ppm kitosan (T2) (iii) 80 ppm kitosan (T3) (iv) 120 ppm kitosan (T4) (v) 100% NPK (F1) (vi) 75% NPK (F2) (vii) 50% NPK (F3) (viii) 25% NPK (F4). 100% NPK didefinisikan sebagai NPK dengan nisbah 120-60-60. Terdapat enam belas gabungan rawatan yang berbeza dan setiapnya direplika sebanyak empat kali. Enam parameter telah direkodkan merangkumi tinggi pokok, jumlah ruas, diameter batang utama, kandungan klorofil ketika pertumbuhan panikel, jumlah berat basah dan jumlah berat kering. Analisis tanah untuk pH, N dan P sebelum dan selepas eksperimen juga telah dijalankan. Semua data dianalisis dengan menggunakan ANOVA pada tahap 5% signifikasi. Keputusan ANOVA untuk tinggi pokok, jumlah ruas, diameter batang utama, kandungan klorofil ketika pertumbuhan panikel, dan jumlah nitrogen tanah tidak menunjukkan perbezaan yang signifikan di antara kepekatan berbeza kitosan dan baja kimia. Interaksi di antara T4 dan F2 menunjukkan perbezaan yang signifikan (231.5 g) terhadap jumlah berat basah pokok. Interaksi tersebut adalah 88% lebih besar berbanding interaksi di antara T2 dan F3 (122.6 g). Interaksi d antara T4 dan F2 menunjukkan perbezaan yang signifikan (44.9 g) sebanyak 64.9% berbanding T1 dan F4 (27.2 g) terhadap berat kering pokok. Untuk pH tanah, interaksi di antara T4 dan F4 menunjukkan nilai yang tertinggi (6.6) dan merupakan 8.6% lebih besar berbanding interaksi di antara T1 dan F1(6.1). Interaksi di antara T4 dan F3 menunjukkan perbezaan yang signifikan terhadap fosforus tanah (0.52 mg kg^{-1}), 92% lebih besar berbanding interaksi T1 dan F2 (0.27 mg kg^{-1}). Keputusan yang diperoleh melalui kajian ini menunjukkan bahawa kitosan dan baja kimia tidak mendatangkan perbezaan yang signifikan terhadap kebanyakan parameter vegetatif padi. Walau bagaimanapun, kesan positif kitosan terhadap tumbuhan tidak dapat diabaikan dan kajian jangka masa panjang perlu dilakukan untuk mengenal pasti potensi sebenar kitosan terutama sekali terhadap hasil padi huma.

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LIST OF SYMBOLS, UNITS AND ABBREVIATIONS

%	Percentage
cm	centimeter
g	gram
ha	hectare
kg	kilogram
m	meter
mL	mililiter
mm	milimeter
L	liter
ppm	parts per million
WAT	weeks after transplanting

CHAPTER 1

INTRODUCTION

1.1 Background of study

Paddy is the staple food crop in Malaysia and is consumed as rice. In 2013, paddy cultivation covers an area of 674,332 hectares throughout the country (Paddy Statistics of Malaysia, 2013). A rise in rice consumption is expected from 2.30 million metric tonnes in 2010 to 2.69 million metric tonnes in the upcoming 2020 due to the increase in population (Department of Agriculture and Agro-based Industry Malaysia, 2011). Hence, paddy production is very important in ensuring the nation's food security.

Two types of paddy are being cultivated and produced in Malaysia, depending on the condition in which it is planted, either wetland or dryland. Dryland paddy refers to strictly rain-fed paddy planted on dry areas including in upland or lowland environments (Greenland, 1997). In 2012/2013 planting session, the total dryland production in Sabah was very low, with 13,130 metric tonnes out of 2,625,845 metric tonnes of the total paddy production on that same year, contributing only 0.5% to the total paddy production (Paddy Statistics of Malaysia, 2013).

The low dryland paddy production may be due to low yield, weed infestation, and pest and disease attack. The most disturbing factor would be the low yield since that farmers depend heavily on the production of their crop to create income. Fertilizers could be applied to rectify this problem. It is common among farmers to use chemical fertilizers in order to increase the yield and production of their crops.

However, only a fraction of the fertilizer applied to the soil is taken up by the crop, the rest either remains in the soil or is lost through leaching, physical wash-off, fixation by the soil, or release to the atmosphere through chemical and microbiological processes. Furthermore, the widely used synthetic fertilizer could hamper soil fertility if used excessively, which to some extent, becomes toxic through the accumulation of non-degradable metals. A significant decrease of NO_3^- leaching could be obtained with reducing nitrogen application rate by 5% less than that of the required rate to achieve maximum yield (Sexton *et al.*, 1997).

Growth enhancers may be used to optimize fertilizers application and increasing yield. Examples are synthetic plant growth regulators, plant growth-promoting bacteria and chitosan. For smallholders, obtaining the synthetic plant growth regulators for their crop may be impossible due to the costly price. Furthermore, the inconsistent response of plant growth regulators in the field due to application rates and timing, as well as environmental conditions at the time of application is a constraint needs to be considered (Ball, 1999). Plant growth-promoting bacteria, on the other hand, which benefits have been widely elucidated, may be scarce due to soil conditions including temperature, moisture and plant numbers and types grown under the soil.

Chitosan is a natural biopolymer which can be obtained and modified from chitin. Chitin is the main structural component of squid pens, cell walls of some fungi and shrimp and crab shells. Chitosan is easily-degradable, with high affinity and non-toxicity, which will bring no harm to the consumers either human or livestock (Boonlertnirun *et al.*, 2008).

Stimulation of plants immunity against bacteria and fungi, as well as improvement of nitrogen transportation in the functional leaves contribute to plant's growth and development through chitosan application (Malek, 2012). These processes affect the yield of a crop, which could be either biological yield or economical yield (S.C. Panda, 2010).

The yield potential of a plant including paddy could be determined by observing the growth performance of the paddy. By promoting the growth performance of the paddy, the yield potential could be estimated and increased. With paddy having three (3) stages of growth, a manipulation in one of the stages by giving positive treatments might have significant differences in term of the potential yield.

1.2 Justification

This research was conducted to study the effect of different concentrations of chitosan and chemical fertilizer application towards the growth performance of the selected dryland paddy, Padi Beruang. Research on dryland paddy has been less intensive compared to wetland paddy, although it is widely grown in the interior parts of the country. Chitosan is identified as an alternative to enhance the growth and potential yield of paddy. However, there is no published references to date on the effect of chitosan towards the growth performance of Sabah dryland paddy. Vegetative growth performance is part of the developmental processes, which results in the total paddy grain yield. Application of chemical fertilizer during vegetative growth may help to increase the yield potential. But, the cumulative effect may bring harm to the soil and future crop establishment. To reduce this effect, organic fertilizers and growth enhancers can be used as an alternative to chemical fertilizer that is heavily used by farmers. Therefore, the relationship between the two factors, chitosan and fertilizer application, must be studied to reduce the usage of chemical fertilizers. It is vital to prove that there are significant statistical differences among the concentrations of chitosan and rates of chemical fertilizers to ascertain the best combination of factors. This could benefit local farmers to optimize the use of chemical fertilizers and to increase yield.

1.3 Objectives

1. To determine the effect of chitosan and NPK fertilizer to the growth performance of the dryland paddy.
2. To determine the optimum rate of chitosan and NPK fertilizers to the growth performance of the dryland paddy.

1.4 Hypotheses

1. H_O: There is no significant difference in chitosan and NPK fertilizers to the growth performance of the dryland paddy.

   H_A: There is a significant difference in chitosan and NPK fertilizers to the growth performance of the dryland paddy.

2. H_O: There is no significant difference on growth performance of the dryland paddy as affected by different concentrations of chitosan and NPK fertilizers.

H_A: There is a significant difference on growth performance of the dryland paddy as affected by different concentrations of chitosan and NPK fertilizers.

CHAPTER 2

LITERATURE REVIEW

2.1 Background

Grain yield and yield attributes are major constraints in the production of dryland paddy in Sabah, contributing less than it could to the self-sustainability level of paddy. For successful rice production, appropriate control of vegetative growth throughout the duration of the crop, suitable transplanting densities for optimum tillering and control of leaf growth by controlling water, fertilizer and chemical inputs are essential for improving the growth variables responsible for high yield.

To increase yield, farmers are putting more fertilizer and chemical inputs. However, it may serve harm to the environment in the long-term. Chitosan, which has been known for its biocompatibility, biodegradability, non-toxicity and adsorption abilities may be incorporated with the widely used NPK fertilizers to solve this problem. Selection on the best interaction between these two factors may help to improve potential yield of the dryland paddy, save cost and reduce excessiveness in dosage used.

2.2 Paddy (*Oryza sativa* L.)

Paddy which is consumed as rice is the most important food crop in Asia. The demand will soar by 2050 especially in the rice-consuming and rice-producing regions of Asia, Africa and America, due to the growth of world's population (Dawe, 2007; Easterling *et al.,* 2007). However, major constraints such as shortage of land and water, accompanied by increases in field demand (Khush, 2005) must be overcome by optimizing production from the current paddy-cultivated lands.

2.2.1 Botany

Paddy is an annual grass of the family Poaceae which bears round, hollow and jointed culms composed of a series of nodes and internodes, flat leaves and terminal panicle. It can grow up to 50 cm to 130 cm depending on the cultivar and growing season. Paddy has fibrous roots consisting of rootlets and root hairs, arising from the base of the shoots.

The rice stem, or better known as the culms, consists of nodes and internodes arranged in an alternate orders. The tillers are branches developed from the leaf axils at each unelongated node of the main shoots. These tillers grow independently by means of its own adventitious roots. Tillering is a two-stage process which includes the formation of axillary buds of each leaf axil and its subsequent growth.

The leaves are borne at an angle of every node and they possess two parts – leaf blade and leaf sheath (Panda, 2010). The uppermost of leaf below the panicle is the flag leaf and the number of leaves on the stem goes on reducing from the main culm to primary tillers and from primary to secondary and then to tertiary tillers.

Inflorescence occurs at terminal panicle with fifty (50) to five hundreds (500) spikelet depending on the cultivar. Single spikelet is borne on a short pedicel. They can be oblong to lanceolate in shape measuring 7 mm to 11 mm long containing single bisexual flower with two small glumes, a large 6 to 10 mm long. It has boat-shaped lemma sometimes with awn up to 15 cm long and similarly, palea with very short awn, six stamens, a broad ovary, and two plumose stigmas. The grain, also known as the fruit or caryopsis is varying in size, shape and color.

The ideotype for a dryland paddy crop should has features including short growth duration (85-100 days), effective deep root system to absorb water in a dryland environment, dwarf (less than 100 cm) with plant having erect leaves and thick stem, early strong fertile tillering, synchronized flowering, good number of panicles at higher density about 400 panicles per square meter, highest number of grains per panicle, moderate seed dormancy, and resistant to insect pests and diseases.

2.2.2 Dryland Paddy

Dryland paddy refers to paddy cultivation which depends solely on rainfall to irrigate the crop, despite of whether it is planted on highland or lowland area (Greenland, 1997). This is consistent with the definition given by IRRI (1984; 1986) in which dryland paddy, also known as upland paddy, is the paddy grown where there are no efforts made to impound water and where there is no natural flooding of the land. Multiple studies have reported that the yield of dryland paddy is generally low due water deficiency, weed competition and nutrient deficiencies as the limiting factors. In Malaysia, research on dryland paddy has been neglected due to the low and unstable grain yield (Mariam *et al.*, 1991).

Despite of the low-yielding properties, it provides subsistence yields even under adverse conditions of the uplands, hence its ability to promote the development of a large track of idle lands in Malaysia (Hanafi *et al.*, 2009). The varieties are drought tolerant but with low yield potential and the tendency to lodging under high levels of external inputs such as fertilizers and supplemental irrigation.

2.3 Growth and Development of Paddy

Depending on the variety and the environment under which it is grown, paddy takes three (3) to six (6) months from germination to maturity. For Padi Beruang, the planting duration is three (3) months until it reaches maturity and ready to be harvested. There are three (3) growth stages of paddy including vegetative stage, reproductive stage, and ripening stage. The vegetative stage refers to the period from a paddy plant germinate up to the initiation of panicle primordia. Reproductive stage refers to the period from initiation of panicle primordia up to heading. Meanwhile, the period from heading to maturity occurs at ripening stage.

The vegetative stage is characterized by active tillering, gradual increase in plant height, and leaf emergence at regular intervals. These properties contribute to the increasing of the leaf area that receives sunlight. Light environment and interception of light strongly influence plant growth and development. Whole-plant growth and competitive ability at a different irradiances are dependent on photosynthetic rate and structure of individual leaves, canopy geometry and dynamics, and biomass allocation among plant components (Givnish, 1988).

2.4 Vegetative Growth of Paddy

Vegetative phase is further divided into a few stages including seedling stage, transplanting stage, tillering stage, and vegetative lag phase. Seedlings depend upon carbohydrate reserve of the grain up to the 15th day after germination or until the first two leaves have come out. Seedling phase would end after fifth leaf has developed and during this period the seedlings exhaust almost entire endosperm.

Transplanting stage covers the period from uprooting, transplanting of seedlings until full recovery following transplanting. The secondary adventitious roots develop within four (4) to ten (10) days after transplanting and they start absorbing nutrients from soil and sustain growth. The root injury due to uprooting of seedlings could be if they are uprooted carefully under moist condition. The transplanting stage took up to two weeks in the nursery.

Tillering stage occurs ten (10) days after seedling establishment to maximum tillering stage which comes between 35 to 42 days after transplanting. Paddy often stop producing tillers after tertiary tillers have been produced. The tiller number declines after the plants have reached the maximum tillering stage. After reaching maximum tillering stage, vegetative lag phase will occur until panicle initiation, in which some of the tillers die because of tiller mortality.

2.5 Growth Phases and Yield Components

Paddy grain yield results from developmental processes that are synchronized with plant growth. The relationship of yield components to plant growth phases can be observed in paddy. Number of panicles per unit area (panicles/m^2) which is one of the yield component is reflecting the plant vigor, tillering, planting density, soil fertility and flood depth of the vegetative phase of paddy.

All potential spikelet will be formed during panicle initiation during the reproductive phase. This is highly associated with number of spikelet per panicle as the parameter of study. Furthermore, this will also affect the percentage of filled grains as one of the yield components since that spikelet development is sensitive to environmental factors, which might preclude grain filling during the next phase (Moldenhauer and Gibbons, 2003).

2.5.1 Factors Determining the Growth and Final Yield of a Crop

The rate of growth and final yield of a crop including paddy depends on many factors. Nutrient supply and hence the response of the crops to the fertilizers is just a part of it. Generally, climatic factors such as rainfall, temperature, solar radiation, interaction between evaporation and evapotranspiration, and climate and insect and disease incidence will affect the growth and final yield of a crop. Furthermore, crop variety, planting density, seedling emergence, and control of pest, disease and weed are also some of the factors included.

In term of soil, the available nutrients, presence of toxic substances, soil physical condition and soil depth would also determine the growth and final yield. Factor such as application of required nutrients, application at correct time(s), placement and application of correct amount of fertilizer would definitely affect the growth and final yield of a crop, including paddy (Gupta and Toole, 1986).

2.6 Chemical Fertilizer

It is inevitable to use chemical fertilizer to get the maximum yield in the cultivation of any crop including paddy. This is to fulfill the nutrients required by the crop. Conventionally, the nutrients or elements are grouped into macronutrients and micronutrients. Nitrogen and potassium are often taken up in the largest amounts, which can exceed 100 kg ha-1 (Wild, 1993). Fertilizer management is one of the most important factors to achieve high yields. Good nutrient management is the key to good harvest (Krishnaprabu, 2013).

2.6.1 Role of Nitrogen

Paddy needs large amounts of nitrogen at the early and mid-tillering stages to maximize the number of tillers. It also requires considerable amount of nitrogen even in the ripening stage. Better stems and larger grains can be obtained through nitrogen application. Supplying nitrogen through ammonia form is preferred since that it is more stable and will not lost through leaching and denitrification compared to the nitrate form of nitrogen (Panda, 2010).

2.6.2 Role of Phosphorus

Phosphorus can stimulate growth and development of the roots, making plants more resistant to drought and facilitates better nutrient absorption. It can also promote early flowering and ripening, and encourage active tillering which enables the crop to recover more rapidly after any adverse conditions encountered in early stage of growth. Furthermore, it also improves the quality of rice owing to the higher phosphorus content of the grains (Panda, 2010).

2.6.3 Role of Potassium

Potassium exerts a favorable influence in tillering and the weight and the size of the grains. Furthermore, it will also renders the crop resistant to diseases and adverse effects of unfavorable climatic conditions by strengthening and stiffening the plant cells. (Panda, 2010).

2.6.4 Effect of Continuous Application of Chemical Fertilizer

Application of balanced NPK fertilizer has undeniably increase paddy yield. However, fertilizers are always associated with residues either acidic or alkaline when applied to the soil. The cumulative effect may bring harm to the future of crop establishment in affected soil. Chang *et al.* (1947) and Rhind and Tin (1948) have both reported this issue where continuous application of chemical fertilizer would decrease the yield through a number of years. With the application of organic fertilizers, the yield is able to be increased again after several years.

Multiple studies also found that residual value of nitrogenous fertilizers on paddy was not at all reliable. Continuous application of chemical fertilizer do not change soil pH, organic carbon and total N. Application of organic fertilizer on the other hand, could increase the organic carbon and total nitrogen content in the soil, hence build up soil fertility (Acharya *et al.*, 1956; Chaudry and Mahapatra, 1963).

2.7 Chitosan

Chitosan, produced by poly (1,4)-2-amino-2-deoxy-β-D glucose, is one of the most abundant natural amino polysaccharides. It is derived from chitin which can be found in exoskeleton of shellfish such as shrimp, lobster or crabs, and in the cell wall of the fungi (Rinaudo, 2006). They are copolymers that can be found together in nature.

Chitosan has wide scope of application on various crops and plants. Known for its biocompatibility, biodegradability, non-toxicity and adsorption abilities, chitosan is an efficient alternative which can be used to improve food productivity, yet at the same time bringing no severe harm or any adverse impact on the ecosystem including the consumers. Its positive charge confers to the numerous and unique physiological and biological properties with great potential in a wide range of industries including agriculture.

Chitosan can serve as plant growth promoter, as being demonstrated in several studies by Khan *et al.* (2003), Chibu and Shibayama (2003), and Gornik *et al.* (2008), in which chitosan has enhanced the plant growth and development used in their respective study, which includes rooting of gravevine cuttings, photosynthesis of maize and soybean, and growth of lettuce, tomato and rice.

2.7.1 Effect of Chitosan on Plant Height

Plant height is an important agronomic trait linked to plant type and yield potential. It is often studied as one of the vital growth components for crops including paddy. According to an experiment done by Boonlertnirun *et al.* (2012), application of chitosan in combination with mixed chemical fertilizer increased plant height greater than the other applications. In a report by Dzung *et al.* (2011), chitosan oligomer strongly increased height of coffee seedlings. Polymeric chitosan at 10, 20 and 40 mg L-1 also significantly enhanced the average plantlet shoot length of hybrid Dendrobium orchids (Pompienpakdee *et al.*, 2010).

2.7.2 Effect of Chitosan on Tillers Number

Among all the yield attributes for paddy, number of tillers is the most yields contributing factor. Tillering and number of grains per panicle were compensating characters and each in turn is affected by the one preceding it. The development of characters was extended or curtailed by the prevalent environmental conditions, seasons, etc. and based upon the extent of development of the preceding characters (Jacobson, 1916).

According to an experiment done by Kananont *et al.* (2015), the application of fermented chitin waste (FCW) led to a significantly higher tiller number, shoot biomass, and grain yield of paddy. In another report by Boonlertnirun *et al.* (2012), the maximum tiller numbers of paddy were obtained from application of chitosan in combination with mixed chemical fertilizer but did not differ from that of mixed chemical fertilizer application. Ramos-Garcia *et al.* (2009) reported that gladiolus corms were accelerated in approximately four (4) days after applying commercial chitosan. Plant number of faba bean were increased after applying water soluble chitosan at 100 ppm (El-Sawy *et al.*, 2010).

2.7.3 Effect of Chitosan on Main Culm Diameter

Lodging in cereal crops often incurred a great deal of damage. Much labor is necessary to harvest the lodged plants. This problem usually occurs when excessive fertilizers, especially nitrogen, are supplied in the hope of gaining much grain. Dryland paddy grew excessively tall when fertilizer was abundant and became susceptible to lodging, resulting in significant yield loss (Okuno, *et al.*, 2014). To date there is no study on the relation of chitosan and culm diameter.

2.7.4 Effect of Chitosan on chlorophyll Content

Technically, the nutrient condition of crop plants at critical stages should be estimated *in situ* to determine the appropriate amount of topdressing. Nitrogen concentration (g kg^{-1}, dry weight basis) of the leaf or whole plant has already been proven to be relevant to many requirements of crop monitoring to assess the nutrient condition of paddy (Shibayama, *et al.*, 2012). Using SPAD meter to assess leaf chlorophyll

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