EFFECTS OF EFFECTIVE MICROORGANISMS ON THE GROWTH PERFORMANCE OF UPLAND RICE UNDER DROUGHT CONDITION

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EFFECT OF EFFECTIVE MICROORGANISMS ON UPLAND RICE UNDER DROUGHT CONDITION

ABSTRACT

A field experiment was conducted at the Faculty of Sustainable Agriculture field, Universiti Malaysia Sabah, campus Sandakan, Sabah to study the effect of effective microorganisms on the growth performance of upland rice under drought condition. In this experiment, the experimental design used was 'Completely Randomized Design' (CRD), and there are four treatments used which are paddy crop planted in Silabukan soil with irrigated condition, paddy crop planted in Silabukan were soil treated with effective microorganisms under irrigated condition, paddy crop planted in Silabukan soil were treated with effective microorganisms under stress condition and lastly paddy crop that were planted in Silabukan soil without effective microorganisms and under stress condition. All of these treatments have five replicates. The parameters measured were the plant height, number of tiller, number of active tiller, soil pH, chlorophyll content, and shoot dry weight. Effective microorganism application was found to show the highest mean for all parameters except for the number of active tiller of the upland rice Paddy variety Mutiara. However, in general the application of effective microorganisms did not have significant effect for all parameters taken.

KESAN EFEKTIF MIKROORGANISMA TERHADAP PRESTASI PERTUMBUHAN PADI HUMA DI BAWAH KEADAAN KEMARAU

ABSTRAK

Eksperimen lapangan telah dijalankan di Fakulti Pertanian Lestari di Universiti Malaysia Sabah, Kampus Sandakan, Sabah untuk mengkaji kesan efektif mikroorganisma terhadap prestasi pertumbuhan padi huma di bawah keadaan kemarau. Dalam eksperimen ini, reka bentuk eksperimen yang digunakan adalah Rekabentuk Rawak Lengkap (CRD), dan terdapat empat rawatan yang digunakan yang pokok padi yang ditanam di dalam tanah Silabukan dengan keadaan pengairan, pokok padi yang ditanam di dalam tanah Silabukan dirawat dengan efektif mikroorganisma di bawah keadaan pengairan, pokok padi yang ditanam di dalam tanah Silabukan dirawat dengan efektif mikroorganisma dengan keadaan tekanan dan akhir sekali ialah pokok padi yang ditanam di dalam tanah Silabukan tanpa dirawat dengan efektif mikroorganisma dan di bawah keadaan tekanan. Semua rawatan ini mempunyai lima replikasi. Parameter yang diukur adalah ketinggian tumbuhan, bilangan anakan, bilangan anakan aktif, pH tanah, kandungan klorofil, dan berat kering pucuk. Aplikasi efektif mikroorganisma didapati mempunyai min yang paling tinggi untuk semua parameter kecuali bilangan aktif anakan padi huma Padi Mutiara. Walau bagaimanapun, secara umum penggunaan efektif mikroorganisma tidak mempunyai kesan yang ketara untuk semua parameter.

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LIST OF SYMBOLS, UNITS AND ABBREVIATIONS

ANOVA	Analysis of Variance
CRD	Completely Randomized Design
EM	Effective Microorganisms
EMAS	Effective Microorganisms Activated Solution
FSA	Faculty of Sustainable Agriculture
g	Gram
ha	Hectare
L	Liter
LSD	Least Significant Difference
m	Metre
Sdn. Bhd.	Sendirian Berhad
SAS	Statistical Analysis System
UMS	University Malaysia Sabah
C	Control
S	Stress
EMS	Effective Microorganisms under Stress
DOA	Department of Agriculture

CHAPTER 1

INTRODUCTION

1.1 Background

Paddy is one of the most important staple foods in Asia, providing average 32% of total calorie uptake (Maclean *et al.*, 2002). In Malaysia, rice is normally cultivated either as wet paddy (Peninsular Malaysia, 503,184 ha) or upland rice (Sabah and Sarawak, 165,888 ha) (DOA, 2005). Upland rice cultivation is practiced mostly by the rural communities living especially in Sabah and Sarawak. It is still an important agricultural activity for home consumption and sometimes the farmers sell their surplus to earn some money. Certain upland rice varieties have desirable characteristics, particularly in terms of their fragrance, colors, sizes, and shapes. These qualities contribute to their popularity among the farmers and health-conscious consumers as an organic food. However, these upland rice varieties have not been commercialized due to their low grain yields. It is reported that research on upland rice has been neglected because of the low and unstable grain yields (Marian *et al.*, 1991).

In the year of 2000 to 2013, the import of rice in Malaysia has increased from 596 200 tons which valued at RM698.3 million to 876 100 tons which valued at RM1.5 billion which is because of the increase of population in Malaysia (MARDI, 2015). Apparently, the increase of this import worrying the government and it is predicted that the import for the following year will still increase as the population grows. Therefore, a solution is needed in order to avoid this consequence and since then, various different approaches have been used to improve the performance of paddy. Malaysian Minister of Agricultural and Agro based Industry Datuk Seri Ismail Sabri

Yaakob said that the Ministry wants Malaysia to achieve full self-sufficiency level (SSL) in paddy production by the year 2020. Therefore, in order for this goal to be achieved, the ministry will spend RM2.2 billion out of its RM6 billion budgets allocated so that the farmers could increase their paddy yield. In order to achieve the target of 100% self-sufficiency level (SSL), the minister has told the farmers to increase their paddy crop yield from current 6 tons per hectare to 7 tons per hectare (MARDI, 2015).

Effective microorganisms play important roles such as increase the number of beneficial microorganisms in soil, thus maintaining the natural ecosystem of the cultivated land and diminishing the risk of environmental pollution with improved crop productivity and quality (Higa, 1991). Effective microorganisms refer to any of the predominantly anaerobic organisms blended in commercial agricultural amendments. The principle of activity of the effective microorganisms is by increasing the bio-diversity of the micro flora increasing the yield of the crop and photosynthetic bacteria are the back bone of the effective microorganisms, working synergistically with other microorganisms to provide the nutritional requirement to the plant and also reduce the disease problem (Anibal *et al.,* 2007).

Meanwhile, scarcity of water is a severe environmental constraint to plant productivity. Faced with scarcity of water resources, drought is the single most critical threat to world food security. It was the catalyst of the great famines of the past (Somerville and Briscoe, 2001). Drought is a meteorological term and is commonly defined as the inadequacy of water availability including period without significant rainfall that affects the crop growth (Hanson *et al.,* 1995). Because the world's water supply is limiting, future food demand for rapidly increasing population pressures is likely to further aggravate the effects of drought (Somerville and Briscoe, 2001). The effect of drought range from morphological to molecular levels and are evident at all phenological stages of plant growth at whatever stage the water deficit takes place (Farooq *et al.,* 2012). Therefore, this experiment aims to study the effect of effective microorganism on the growth performance of upland rice under drought condition.

1.2 Justification

Paddy is one of the most important staple foods grown in Malaysia. As the population increases, the demand is also increases. It is suggested that effective microorganisms can improve productivity and quality of crop while maintaining the natural ecology and does not harm the environment (Higa, 1991). This effective microorganism may increase the productivity of rice as well as rice quality. As the land is limited, some farmers tend to increase the fertilizer application rate in order to increase the yield. Some also used synthetic fertilizer to increase the rice productions which lower the rice quality and harming the environment of the cultivated land. Thus, using environmental friendly method as in the application of effective microorganisms may give better result. Crop production also is decreased by extreme drought stress that weakened the plants and makes it more susceptible to pest and disease which resulted in yield loss. Currently, there are no economically viable technological means to facilitate crop production under drought. Therefore, this experiment aims to determine the effect of effective microorganisms on the growth performance of rice under drought condition.

1.3 Objective

The objective of this experiment was to determine the effect of Effective Microorganisms (EM) on the growth performance of upland rice under drought condition.

1.4 Hypothesis

The hypothesis is as follow:
H_O: The application of effective microorganisms does not have significant effect on the growth performance of upland rice under drought condition.

H_a: The application of effective microorganisms has significant effect on the growth performance of upland rice under drought condition.

CHAPTER 2

LITERATURE REVIEW

2.1 Paddy Botany

Paddy is a grass plant that belongs to the family Gramineae, subfamily of Oryzoideae, tribe of Oryzeae and genus of Oryza. There are two cultivated species of paddy; *Oryza sativa* (L) and *Oryza glaberrima* (steud). *Oryza sativa* is widely grown in most part of the world including Asia, parts of Europe and America whereas *Oryza glaberrima* is restricted in Africa. Chinese legends attribute the domestication of rice to Shennong, the legendary Emperor of China and inventor of Chinese agriculture (Yang, 2005). Genetic evidence has shown that rice originates from a single domestication 8,200–13,500 years ago in the Pearl River valley region of China (Huang *et al.,* 2012).

From East Asia, rice was spread to Southeast and South Asia (Liu *et al.,* 2012). Rice was then introduced to Europe through Western Asia, and to the America through European colonization.The cultivated species of *Oryza sativa* can be further divided into three subspecies; indica, japonica and javanica based on the morphological and physiological characteristics. *Oryza sativa* is a diploid species with 24 chromosomes (2n=24).

The cultivated paddy is generally characterized as a semi-aquatic annual grass with round, hollow, narrow and jointed culms together with rather flat leaves and sessile leaf blades and a terminal panicle. The roots are fibrous with rootlets and root hairs. At maturity, the paddy plant has a main stem and a number of tillers. The paddy culms (stem) consist of a series of nodes and internodes arranged in alternate orders. Tillers which are the side shoots are produced from the basal nodes on the main culms known as primary tillers which give out secondary tillers and then secondary tillers branch into tertiary tillers. The leaves consist of blade and the leaf sheath which wraps the culms and are borne at an angle of every node. The uppermost leaf below the panicle is known as flag leaf and the number of leaves on the stem decreases from main culms to primary tillers and to secondary tillers and then to tertiary tillers. Each panicle has spikelets (grains).

The grain, known as caryopsis is a dry one-seeded fruit, with its pericarp fused with the seed coat. The seed consists mainly of husk, pericarp, endosperm and embryo. The surface contains several thin layers of differentiated tissues that enclose the embryo and endosperm. The cotyledon (scutellum) is a fleshy, shield-shaped which provide food for the germinating embryo (Panda, 2010).

The face of the scutellum is differentiated into a columnar epithelium with elongated cells in contact with endosperm which absorb food from the cotyledon. A poorly developed vascular system consisting of procambial stands is at the middle of the scutellum. The endosperm made up the major part of the seed. The aleurone layer serves as the outer layer of the cells of the endosperm and in cross-section, these cells are irregularly hexagonial to polygonal. The protein of the seed mostly contained in the aleurone layer. A paddy grain weighs about 10 to 45 mg at 0% moisture content. The grain length, width and thickness vary widely among varieties. The husk weight constitutes about 20% of the total grain weight (Panda, 2010).

2.2 Paddy Growth and Development

The growth of paddy can be physiologically divided into three phases; vegetative phase, reproductive phase and ripening phase (Panda, 2010).

2.2.1 Vegetative Phase

Vegetative phase is characterized by a gradual increase in plant height and leaf emergence at regular intervals. It is divided into four stages known as seedling stage, transplanting stage, tillering stage and vegetative lag phase. Seedling stage begins when the seed germinates into young seedling with seminal and lateral roots and green leaves. The grain serves as the source of carbohydrate for the seedling up to 15 days until the first two leaves emerge. This phase ends after the fifth leaf has developed and the entire endosperm is used up (Panda, 2010).

The transplanting stage includes the period of uprooting, transplanting of seedling until full recovery of the transplant. The formation of second adventitious root within 4 to 10 days after transplanting begins to absorb nutrients in the soil and sustain the growth. Transplant shock can be minimized if the seedlings are uprooted carefully with the presence of water (Panda, 2010). The vegetative growth stage can be reduced for direct seeded paddy.

Tillering stage involves pre-tillering which is the period when seminal roots further develop, the secondary roots develop and the first four leaves appear. Tillering usually occurs after the fifth leaf stage when the first tiller is visible and emerges from the axillary bud on the culm. The plant stops producing tillers after tertiary tillers and the number of tiller declines after it reaches maximum tillering stage.

The period from the end of tillering stage to beginning of reproductive stage is known as vegetative lag phase. In this stage, the height and stem diameter increases at a slower rate while the tiller number decrease due to tiller mortality (Panda, 2010). However, this stage is not noticeable in early maturing varieties but it occurs in long duration plant.

2.2.2 Reproductive Phase

Reproductive phase begin with panicle initiation stage which takes place approximately 21 to 25 days before heading. The internal auxin concentration accelerates the elongation of the internodes as soon after panicle initiation stage. This is known as booting stage. The emergence of panicle tip takes place after the booting stage is over. This heading stage takes about 15 days after booting in all varieties (Panda, 2010). After 20 to 25 day from booting, flowering occurs. It continues successively until all spikelets in the panicle bloom. This is followed by pollination and fertilization.

2.2.3 Ripening Phase

This phase takes about 25 to 35 days after the paddy undergoes the following stages; milky stage, soft dough stage, hard dough stage and maturity stage. The water content in the grain turns milky between 7 to 12 days, the starch in the grain begin to become firm but soft and later turn into hard dough which takes about 2 to 3 weeks after milky stage. Finally, the grains turn hard, clear and ready to harvest. The moisture content is approximately 20% to 22%.

2.3 Effective Microorganisms (EM) Background

Effective Microorganisms were developed at the University of the Ryukyus, Okinawa, Japan, in the early 1980s by Prof. Dr.Terou Higa. Effective microorganism (EM) is a commercial bio-fertilizer that contains a mixture of co-existing beneficial microorganisms collected from natural environments. Predominantly it consists of species of photosynthetic and lactic acid bacteria, yeast, and actinomycetes. The expansion process of EM Technology began in 1989 with the inception of the 1st International Kyusei Nature Farming Conference in Thailand, where the need to scientifically validate this technology and to enhance its use was discussed. Consequently, Asia Pacific Natural Agriculture Network (APNAN) was founded. It included 13 countries ranging from the west coast of the USA through Asia to Pakistan (Hussain *et al.*, 2000).

EM is a fermented mixed culture of naturally occurring species of co-existing microorganisms in acidic medium (pH below 3.5). Among the main microorganisms in EM culture are the species of photosynthetic bacteria (*Rhodopseudomonas plastris* and *Rhodobacter sphacrodes*), lactobacilli (*Lactobacillus plantarum, L. casei,* and *Streptococcus lactis*), yeasts (*Saccharomyces spp.*), and actinomycetes (*Streptomyces spp.*) (Arshad *et al.,* 2011).

2.4 Effect of EM on crop

It is suggested that EM increase the number of beneficial microorganisms in soil, thus maintaining the natural ecosystem of the cultivate land and diminishing the risk of environmental pollution with improved crop productivity and quality (Higa, 1991). EM is known to solubilize and make available the nutrients required by crop plants and suppress the infestation of harmful microorganisms and improve soil texture by increasing the humus content (Higa, 1991). Microorganisms in EM improve crop health and yield by increasing photosynthesis, producing bioactive substances such as hormones and enzymes, accelerating decomposition of organic materials and controlling soil-borne diseases (Hussain *et al.,* 2002).

Numerous field and green house trials are indicative of the benefits of this technology for crop production, as a pro-biotic in poultry and livestock rations, and to enhance the composting and recycling of municipal/industrial wastes and effluents (Hussain *et al.,* 1999). Previous studies on EM application have revealed that plant growth in EM applied treatments was just as good or better, and quality of plant products was superior to conventional farming (Daly and Stewart 1999; Iwaishi, 2000; Javaid, 2006, 2009; Khaliq *et al.,* 2006). However, experiences of some researchers revealed that the effect of EM on crop yield was usually not evident or even negative particularly in the first test crop (Daiss *et al.,* 2008; Javaid *et al.*, 2008, Javaid and Shah, 2010).

It is often difficult to establish the predominance of effective microorganism cultures in soil with only a single application and during only one season. Indigenous soil microbial populations are often constraints to the establishment of these microorganisms (Bajwa *et al.,* 1995). However, these constrains can be overcome through periodic repeated applications of effective microorganisms at least during the first few years. Efficacy of effective microorganisms is also affected by soil types. Furthermore, source and amount of soil nutrients as well as test crop may affect the establishment and efficacy of these microorganisms when application of these microorganisms is started in a soil for the first time (Bajwa *et al.,* 1999, Javaid *et al.,* 2002; Javaid, 2010).

An increase in wheat and rice grain yield was found when EM application was carried out in combination with farmyard manure or mineral NPK (Hussain *et al.,* 1999). The higher grain yield in the present and earlier studies, when EM was applied in combination with organic matters, can be attributed largely to the activity of the introduced beneficial microorganisms, which enhanced the decomposition of organic materials and the release of nutrients for plant uptake (Hussain *et al.,* 1999). However, the fact that EM also increased grain yield when applied with recommended dose of

NPK fertilizers suggests that EM may have induced other mechanisms that exert a positive effect on the yield (Higa and Widdana, 1991).

The enhanced crop growth and yield can possibly be attributed to activity of photosynthetic bacteria such as *Rhodopseudomonas palustris* and *Rhodobacter sphaeroides* present in EM solution. These bacteria are a group of independent, self-supporting microbes. They synthesize useful substances from secretions of plant roots, organic matter and harmful gases such as hydrogen sulfide, by using sunlight and the heat of soil as sources of energy (Kim *et al.,* 2004). The useful substances produced by these bacteria include amino acids, polysaccharides, nucleic acids, bioactive substances, and sugars, all of which promote plant growth and development. The metabolites developed by these microbes are absorbed directly by plants.

2.5 Drought

Drought is a meteorological term and is commonly defined as the inadequacy of water availability including period without significant rainfall that affects the crop growth (Hanson, *et al.,* 1995) and soil moisture storage capacity and it occurs when the available water in the soil is reduced and atmospheric conditions cause continuous loss of water by transpiration or evaporation.

Drought can also best be thought of as a condition of water shortage for a particular user in a particular location. Drought is a gradual phenomenon. Although persistent drought may be characterized as an emergency, it differs from typical emergency events. Most natural disasters, such as floods or forest fires, occur relatively rapidly and afford little time for preparing for disaster response. Droughts occur slowly, over a period of time. There is no universal definition of when a drought begins or ends. Impacts of drought are typically felt first by those most reliant on annual rainfall ranchers engaged in dryland grazing, rural residents relying on wells in low-yield rock formations, or small water systems lacking a reliable water source. Drought impacts increase with the length of a drought, as carry-over supplies in reservoirs are depleted and water levels in groundwater basins decline.

According to the authors of the Intergovernmental Panel on Climatic Change, the temperature increase in the 20th century was assessed as 0.74°C with the steadily increasing rate. Model simulations suggest that an average increase in temperature of up to 2.5-5.4°C can be expected by year 2100 coupled with a decrease in precipitation of about 15% (Ciscar, 2012; Tadross *et al.,* 2009). Under climate of south-eastern

Australia, it was predicted that every 1°C increase in air temperature will cause a 1.5°C increase of surface soil temperature (Ooi *et al.,* 2012). Under field conditions, water shortage often occurs concurrently with high air temperature (say > 30°C in the low to mid-latitudes) and are threat limitations to plant growth (Farooq *et al.,* 2012) and sustainable agriculture (Ahuja *et al.,* 2010). Increasing frequency of water deficits, events of heat waves, and intra- and inter-seasonal variations as well as an increase in the atmospheric carbon dioxide concentration will add another layer of complexity to the effects of drought and heat stresses (Sekhon *et al.,* 2010). The heat wave effects can be anticipated to strengthen as the temperature progressively increases (Battisti and Naylor, 2009).

2.5.1 Effect of Drought on Plants

A period of dry weather, injurious to crops, is often defined as drought that is related to changes in soil and meteorological conditions and not with plant and tissue hydration. Drought stress occurs when the humidity of the soil and the relative air humidity are low and the ambient temperature is high. Predisposition of plants to maintain a high potential of water in the tissues under drought is called dehydration avoidance, and tolerance that determines plant predisposition to survive water deficiency is called drought resistance (Blum, 2005; Vadez *et al.,* 2011).

Drought is an important threat limitation to plant growth and sustainable agriculture worldwide. Plants are frequently exposed to drought that reduces crop yield worldwide. The combined effect of both heat and drought on yield of many crops is stronger than the effects of each stress alone. Agricultural water deficit arises from both insufficient rainfall and soil water during the growing season to sustain a high crop yield (Sekhon *et al.,* 2010; Vadez *et al.,* 2011). Projections show an increase in intense rain events and at the same time reduction in the number of rain days that leads to increased risk of drought (Vadez *et al.,* 2011). Molecular biologists often report the effect of an exotic gene towards drought tolerance and advertise its expected value in breeding (Blum, 2005).

Drought stress tolerance is seen in almost all plants but its extent varies from species to species, even within the species. Water deficit and salt stresses are global issues to ensure survival of agricultural crops and sustainable food production (Jaleel *et al.,* 2007). Conventional plant breeding attempts changed over to use physiological selection criteria since they are time consuming and rely on present genetic variability

(Zhu, 2002). Plant response to drought and heat stress differs in C3 such as wheat and C4 such as maize plants. C4 plants are more sensitive to water deficit due to stomatal closure and reduction of the photosynthetic enzyme (Alfonso and Bruggemann, 2012). However, the effect of high temperature on the photosynthetic capacity is stronger with C3 than C4 plants due to different energy distribution and activities of carbon metabolism enzymes, particularly of rubisco (Salvucci and Crafts-Brandner, 2004).

The decrease in the duration of developmental growth phases caused by heat and drought stresses is partly responsible for yield reduction of cereals by reduction in light interception over the shortened life cycle (Barnabas *et al.,* 2008). Effects of drought and high temperature were reflected in reduced accumulation in plant mass, shorter first internode, increased tillering, early senescence and premature death, and fruit discoloration and damage in various plants (Vollenweider and GunthardtGoerg, 2005; Zlatev and Lidon, 2012).

Drought stress is very important factor for plant growth and affects both elongation and expansion growth. Water deficit is one of the most environmental stresses affecting agricultural production and productivity around the world and may result in considerable yield reduction.

2.5.2 Effect of Drought on Rice

Among the crops, rice is probably more susceptible to drought as compare to other crops. Water stress reduces the leaf area, cell size and intercellular volume (Kramer, 1969). Drought has also been recognized as the primary constraint to rainfed rice production (Datta *et al.*, 1975). A variety that is more resistant to water flow from the stomata into the atmosphere is considered as good for drought tolerance. The reduction in soil moisture may have led to lower water content in the leaves causing guard cells to loose turgor pressure and hence the size of stomatal pores are reduced (Tezera *et al.*, 2002) and/ or causing stomatal closure. In addition, increased stomatal resistance may have led to reduced water transport in the leaves further causing a decrease in stomatal conductance.

Reduction in stomatal conductance decreases transpiration by closing of the stomata, resulting prolong the plant survival by extending the period of availability of essential soil water reserves in the root zone. Stomatal closure also helps to maintain high leaf water content and thereby a higher leaf water potential, which leads to a reduction in photosynthetic activity (Hsiao, 1973). In rice, leaf rolling character and death of leaves are good criteria found useful in assessing levels of drought tolerance in a large scale screening (Chang *et al.,* 1974).

Leaf of any crop plant frequently rolls when plants are suffering from water stress condition. When leaf temperature is increases, the stomata become close and transpiration rate decreased sharply with leaf rolling (Sobarado, 1987). Leaf rolling scored visually in rice either in the morning or mid day. Delay leaf rolling is used as an important selection criteria for drought tolerance in rice, which could be improved by incorporating the gene(s) into those lines/ varieties, that perform better under irrigated condition but not well under water stress condition. A plant having the characteristics of delay leaf rolling under water stress and faster recovery rate after removing the water stress in rice (Singh and Mackill, 1991) was considered as good trait because flag leaf in rice crops plays the important role in grain filling and development (Evans *et al.*, 1975).

CHAPTER 3

METHODOLOGY

3.1 Study Location

This study was conducted in a rain shelter no. 10 (5° 55′ 56.8″ N, 118° 0′ 13.3″ E) which located at Faculty of Sustainable Agriculture (FSA), Universiti Malaysia Sabah (UMS).

3.2 Study Duration

This study was carried out in June 2016 and ends in November 2016 which is about five months.

3.3 Materials and Site Preparation

3.3.1 Rain Shelter Preparation

Rain shelter was cleaned, sanitized and covered with transparent plastic materials to ensure the rain shelter free from pests and diseases and other environmental disturbances from entering into it. Signboard and pots were put inside the rain shelter.

3.3.2 Pot Preparation

Twenty pots with 30 cm in height and 30 cm in diameter from the farm laboratory were prepared and cleaned.

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