Role of Herbicides on Soil Microbial Community Structure and Yield Performance of T. Aman Rice

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*This is to certify that thesis entitled, "**Role of herbicides on soil microbial community structure and yield performance of T.Aman Rice** " submitted to the Faculty of Agriculture, Sher-e-Bangla Agricultural University Dhaka, in partial fulfillment of the requirements for the degree of **MASTER OF SCIENCE in SOIL SCIENCE**, embodies the result of a piece of bona fide research work carried out by **TANJINA AKTER**, Registration No. **11-04314** under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma.*

I further certify that such help or source of information, as has been availed of during the course of this investigation has been duly acknowledged.

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TO

MY

BELOVED

PARENTS

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Dhaka, Bangladesh
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ABSTRACT

The experiments were conducted in Agronomy research field and microbiology laboratory, BRRI Gazipur during the period from July 2020 to November 2020 (Aman season) to study the application of herbicide which shifts soil microbial community structure and yield performance of T. Aman Rice. The experiments were conducted with Randomized Complete Block Design (RCBD) with three replications in field condition and Completely Randomized Design (CRD) in laboratory condition. Treatment comprised of three herbicides viz. (a) $\mathbf{T_1}$ = Pre-emergence, Pendamethalin33EC (b) $\mathbf{T_2}$= Post-emergence, Phenoxlum 20EC (c) $\mathbf{T_3}$ =Ethoxysulfuron (d) $\mathbf{T_4}$ = Control (no herbicide). The unit plot size was 9.6/ m^2. The popular Aman variety, BRRI dhan75 was used as test crop. Urea, TSP and MOP were applied following recommended fertilizer rate. No weeding was done in control plot. Soil samples (0-15cm) were collected from herbicide treated plot at 3, 7, 10, 20, 30 and 60 days after herbicide application (DAHA) for determination of soil microorganisms. The results of the experiment revealed that numbers of microorganisms in rice cultivating soil were initially reduced with the application of these herbicides but their numbers could be recovered 10-30 days after the applications. In case of Pendamethalin 33EC, fungus could be recovered their numbers 7 days after its application, Nitrogen Fixing Bacteria (NFB) & Phosphate Solubilizing Bacteria (PSB) could be recovered 20 days after its application and total bacteria could be recovered 30-60 days after its application. In case of Phenoxlum 20EC, NFB & total bacteria could be recovered their numbers 10 days after its application whereas PSB & fungus could be recovered their numbers 20 days after its application. In case of Ethoxysulfuron, NFB, total bacteria & fungus could be recovered their numbers 20 days

after its application whereas PSB could be recovered their numbers 10 days after its application. Increasing trend of total nitrogen, nitrogen fixing bacteria, phosphate solubilizing bacteria and fungus were found in control plots from 0 day to 60 days. The highest yield (4.35 t/ha) was obtained from Pendamethalin 33EC treated plots compared to control.

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ABBREVIATIONS

Abbreviations	Full word
%	Percent
@	At the rate
AEZ	Agro-Ecological Zone
CFU	Colony Forming Unit
BRRI	Bangladesh Rice Research Institute
cm	Centi-meter
NFB	Nitrogen Fixing Bacteria
m	Meter
et al.	And others
etc	et cetera
g	Gram
PSB	Phosphate Solubilizing Bacteria
SAU	Sher-e-Bangla Agricultural University
Var.	Variety
NA	Nutrient Agar
ai	Active ingredient
PDA	Potato Dextrose Agar
t	ton
DAHA	Days After Herbicide Application
CRD	Completely Randomized Design
RCBD	Randomized Complete Block Design
NBRIP	National Botanical Research Institutes Phosphate
ha	hectre
mg/l	Milligram/litre

Chapter I

Introduction

CHAPTER I

INTRODUCTION

Rice belongs to the Gramminae family with the scientific name *Oryza sativa L*. Rice is the staple food of more than half of the world's population more than 3.5 billion people depend on rice for more than 20% of their daily calories. Rice provided 19% of global human per capita energy and 13% of per capita protein (FAO, 2004). In Asia, rice consumption is very high, exceeding 100 kg per capital annually in many countries. For about 520 million people in Asia Rice is the most important source of the food energy for more than half of the human population. Rice sector contributes one-half of the 7 countries (UAE-FAO, 2012). In worldwide, 474.86 million metric tons of rice was produced from 159.64 million hectares of land with an average yield of 4.43 t/ha during the year of 2014-15 (USDA, 2015). USDA estimates Bangladesh has to produce around 34.51 million tons of rice from an estimated 11.7 million hectares of land in the year 2016-17.

Rice is the agricultural GDP and one-sixth of the national income in Bangladesh (Rai, 2006). According to the Food and Agriculture Organization (FAO) of the U.N., 80% of the world rice production comes from staple food of about 135 million people of Bangladesh. Bangladesh about 75.61% of the total cropped area and over 80% of the total irrigated area are planted rice (BBS, 2013). Food shortage was one of the major problems here due to over population and low yield of food crops. To reach the goal, it was necessary either to increase the crop area or to increase yield/unit area. But due to high population pressure, horizontal expansion of land was not possible. So, increasing yield/unit was the only means. Variety itself is a genetic factor which contributes a lot in producing yield components and yield of a particular crop. Yield components such as number of effective tillers/ hill, number of grains/panicle and weight of individual grain contribute to increase or decrease the yield. Weeds are the major source of yield loss in upland rice and its control is labor intensive. The climate as well as the edaphic condition of Bangladesh is favorable for the growth of weeds. Among the biotic factors, weed is considered as one of the noxious enemies of crop wasting economic inputs worldwide. It is the most acute pest in agriculture with an estimated annual loss of around 40 billion USD per year worldwide (Monaco et al., 2002). Among the harmful pest, weeds contribute maximum losses in crop production, which may potentially reduce crop production by 34%, followed by animal pests (18%) and pathogens by 16% (Abbas et al., 2018). Weeds also rank first in reducing yields (34%) of major crops world-wide (Jabran et al., 2015). On average 25 and 5% production loss is caused by weeds in developing and developed countries, respectively; despite of any weed control measures (Koch, 1992). Rice yield reduction in Bangladesh is 70–80% (direct seeded Aus rice), 30–40% (transplanted Aman rice) and 22–36% (modern Boro rice) (Mamun, 1990; BRRI, 2008).

Weeds compete with crops for nutrients, space and water and thus reduce crop yield. Weeds also exert allelopathic effect on the growth of rice plant (Ismail and Siddique, 2010). Weeding has a great influence on the performance of the associated crop. Salam et al. (2014) obtained higher yield of Aman rice using a pre-emergence herbicide + one hand weeding at 35 DAT. Thus the best weeding needs to be adopted by the farmers with a view to reducing weed infestation and maximizing rice yield. Proper weed management ensures higher yield. Weeding keeps the land clean and soil becomes well aerated and this facilitates the absorption of more nutrients, moisture and higher reception of solar radiation for better growth and yield of rice. The present study was therefore, undertaken to observe the effect of variety, different weeding regimes and interaction effects of variety and different weeding regimes on the performance of transplanted Aman rice.

Weeds compete for the natural resources e.g., light, water, space, and nutrient thereby reduce the crop growth, yield and quality (Ashiq and Aslam, 2014). Rao (2000) stated that an increase in one kilogram of weed biomass corresponds to a reduction in one kilogram of crop dry matter. The farmers of Bangladesh mainly practice conventional (manual i.e. hand pulling through niri or mechanical through rice weeder) methods of weeding, which are laborious and time consuming. However, these methods are now become difficult because of labour crisis at the peak period, which results in severe yield loss (Hasanuzzaman et al., 2009; Rashid et al., 2007). Therefore, farmers of the country are now going to be solely dependent on herbicidal weed control, which is quick, cost-effective and less laborious. As a result, the use of herbicides in the country has been 37-fold increase in the last three decades (BBS, 2017). However, uninterrupted use of the same herbicides for a long period may cause environmental hazards (Aktar et al., 2009, Islam and Kato-Noguchi, 2014), develop herbicide resistant weed biotypes (Heap, 2018), and cause shifts in weed flora (Holt, 1994). Despite such unwanted after effects, no viable alternative is currently available to shift the chemical dependence for weed management in rice.

Herbicidal weed control is now considered as a viable alternative or supplement to conventional weeding (Mahajan et al., 2009; Chauhan and Johnson, 2011; Anwar et al., 2012). However, efficacy of an herbicide mainly depends on its ability to produce a desired effect on the target weeds. In addition, application time of herbicide is also very crucial with respect to its efficacy. Some other researchers stated that integration of more than one weed control methods i.e. integrated weed management is the best for efficient weed control due to its less effects on non-target organism, and for sustainable crop production (Singh et al., 2008; Jabran and Chauhan, 2015; Abbas et al., 2018). Therefore, with a view to enhancing the proper crop growth for getting maximum grain yield by minimizing the crop-weed competition, efficient weed management practices should be practiced. Besides, the selection of appropriate variety is also essential for obtaining a good harvest because different varieties may vary in their canopy structure and growth characteristics and in turn influence the weed-suppressive ability (Mishra and Bhan, 1997; Rahman et al., 2017; Arefin et al., 2018). It has been reported that transplant Aman rice variety may vary for their yield and quality due to different management aspects (Hossain et al., 2008; Sarkar et al., 2014). But, the information regarding the performance of transplant Aman rice varieties of Bangladesh to various weed management practices are scarce in the literature.

Herbicide usage, which was earlier confined to plantation crops, has now expanded to crops like wheat and rice which constitute about 42 and 30% of the total consumption of herbicides, respectively (Yadaraju and Mishra, 2002). The addition of herbicides can cause qualitative and quantitative alterations in the soil microbial populations and their enzyme activities (Min et al., 2002; Saeki and Toyota, 2004). Herbicide application may also kill species of bacteria, fungi and protozoa that combat disease causing microorganisms, thereby upsetting the balance of pathogens and beneficial organisms and allowing the opportunist, disease causing organisms to become a problem (Kalia and

Gupta, 2004). In addition, change in the soil microflora has been listed as one of the possible causes of productivity decline in rice cropping systems (Reichardt et al., 1998).In this paper the effect of pre-emergence herbicide pendamethalin 30 EC together with 2 post-emergence herbicides Phenoxlum 20EC and Ethoxysulfuron on soil microorganisms cropped with BRRIdhan75 is presented.

Objectives:

1. To characterize the herbicide-induced responses of microorganisms in transplanted Aman rice.
2. To evaluate the herbicide-induced tolerance of soil microbes
3. To examine the effect of herbicide on yield performance of T. Aman rice

Chapter II

Review of literature

CHAPTER II

REVIEW OF LITERATURE

Soil is an important component for food production, shelter and fiber for mankind. According to many plant physiologists, the soil is the major source of plant nutrients, but soil quality is necessary for agricultural production and quality is improved by soil bacteria, fungi and protists . The microbial biosphere is the largest pool of biodiversity on earth. In other words, microorganisms can be considered as soil machinery to recycle to the nutrients. The quality of soil and its maintenances can be improved by soil microbes within the soil system. The breakdown of organic matter like animal remains and plant remains will be well-ordered by soil microorganisms, the formation of soil structure, and the rate of biogeochemical cycling is also controlled by soil microbes in the soil. However, soil microorganisms are greatly influenced by several factors including the application of herbicides (Pampulha *et al.*, 2007) which are applied in modern agricultural practices to obtain optimum crop yields (Zabaloy *et al.*, 2008). If microorganisms are sensitive to particular herbicide, its application will interfere with vital metabolic activities of microbes (Oliveira and Pampulha, 2006), thus affect the availability of nutrients in soil (Nautiyal, 2006). The most frequent application of herbicides occurs in row-cropping system, where the herbicides are applied before or during sowing to maximize crop productivity by minimizing other vegetation. Herbicides are introduced as pre-or post-emergence weed killer (Ayansina and Oso, 2006) such as pendamethylin, phenoxlum, ethoxysulfuron.

Extensive studies of the regulatory effects of pre and post emergence herbicides on various crops have been carried out worldwide by different workers and scientists. Some of the related reports are reviewed below:

Habimana Sylvestre and K.N. Kalyanamurthy (2018) carried out a field experiment to study the effect of different weed management practices on soil microbial population, soil chemical properties, available soil nutrients and biological yield of aerobic rice.. The results showed that there was no ill effect from different weed management treatments on the soil microbial population viz., fungi, bacteria and actinomycetes after harvest of rice. The reduced weed density and dry weight in the superior treatments reduced the loss of nutrients from soil thus helped in improving the soil available nutrient status after harvest. All weed management practices in combination with herbicides registered markedly higher biological yield over the weedy check.

Alaa El-DeinOmaral and Ahmed El-Ghandor (2018) carried out an experiment to investigate the effect of two herbicides, thiobencarb 50% EC and penoxsulam 24% SC at their recommended field rates on soil microbial population at regular intervals i.e. 4^{th} to 28^{th} day after treatment, control of weeds and crop yield of transplanted rice. Application of penoxsulam recorded the lowest value of *Echnochlo crus-galli, Echnochlocolona* and total weeds dry weight and achieved the highest number of panicles and grain yield as compared to weedy check, which gave the highest value of total weeds dry weight and the lowest rice yield characters. Penoxsulum was observed to be less toxic than thiobencarb against microbial enumeration, control of weeds and increase crop yield of rice plant.

J. Ferdous and M. S. Hossain (2016) carried out a field experiment to study the effect of weed management practices on the performance of transplanted Aman rice varieties. The results reveal that varieties should significant variation on plant height, number of total tillers/hill , number of effective tillers/ hill , number of non-effective tillers/hill , panicle length, number of grains/panicle, number of sterile spikelets/panicle, 1000-grain weight, grain yield, straw yield and harvest index. Grain yield was the highest in BRRIdhan39. Weeding regime had also significant effect on all the studied crop parameters except 1000-grain weight. The highest grain yield was obtained from weed free condition followed by hand weeding at 15 and 35 DATs. Interaction between variety and weeding regime had significant influence on all the studied crop parameters except 1000-grain weight. The highest grain yield was obtained from the interaction of BRRI dhan39 × weed free condition which was statistically identical (5.50 t/ha) with interaction of variety BR11 × two hand weedings at 15 and 35 DATs. Therefore it may be concluded that BR11 rice could be cultivated using two hand weedings at 15 and 35 DATs for obtaining higher yield.

Santosh Kumar Dubey *et al*.(2018) conducted a field experiment to study the effect of herbicides on soil microbial population of direct-seeded rice. The results revealed that the herbicides, viz. Pendamethalin and Pyrazosulfuron as pre-emergence and Almix, Ethoxysulfuron, Bispyribac-NaandAzimsulfuron as post-emergence were safe for soil microbial populations at recommended rate in direct-seeded rice.

A.K.M Mominul Islam *et al* (2018) conducted a field experiment on aromatic rice varieties. This experiment was carried out by using five different aromatic varieties: Kalijira, BRRIdhan34, BRRIdhan37, BRRIdhan38 and Binadhan13, and six different weed management practices comprising no weeding, weed free, mechanical + manual weeding, pre–emergence herbicide + manual weeding, post–emergence herbicide + manual weeding and pre– + post–emergence herbicide. Among the weed control treatments, application of pre–+ post emergence herbicides offered the highest reduction in weed density and biomass at all sampling dates. The variety BRRIdhan38 gave the highest grain yield (3.4 t/ha) due to the highest performance of the yield contributing characters among the varieties. In interaction, BRRIdhan38 with pre–+ post–emergence herbicide application gave the highest yield (3.4 t/ha) apart from the weed free treatment.

M. S. A. Faruk and M. G. Rabbani (2013) conducted an experiment at the Agronomy Field Laboratory, Bangladesh Agricultural University (BAU), Mymensingh during the period from July to December 2012 to find out the effect of herbicide Prechlor 500 EC on weed control and performance of transplant Aman rice. The highest grain yield (3.62 t/ ha) was obtained from variety BRRIdhan41 with Prechlor @ 1.5 L/ha. The results suggest that farmers can be advised to use herbicide Prechlor @ 1.5 L/ha to boost up the production of BRRIdhan41 controlling weeds during Aman season under the agro-climatic condition of the study area.

Rukinderpreet Singha and Guriqbal Singh (2020) conducted an experiment to investigate the effects of herbicides pendamethalin and imazethapyr and mixture thereof on the growth Rhizobium and Strenotrophomonasin vitro and on soil microflora (bacteria, fungi and actinomycetes) at field conditions. The tolerance of Rhizobium and Stenotrophomonas to pendamethalin at 180–1980 mg/L and imazethapyr at 26–200 mg/L and mixtures thereof in nutrient media was tested. He showed that the tolerance for pendamethalin was higher than for imazethapyr. Pendamethalin and its mixture with imazethapyr did not influence the population of fungi and actinomycetes in rhizosphere (0–10 cm soil depth) at 35 days after sowing and at harvest. Imazethapyr treatments recorded inhibitory effects on bacterial population in rhizosphere at 35 days after sowing but no adverse effect was observed at harvest. Pendamethalin alone and in mixture with imazethapyr were found safe to investigated microorganism groups.

Ramirez, J.G., and Plaza, G (2015) conducted an experiment to evaluate the effect of post-emergence herbicide control programs made by farmers on weed communities and commercial lots of rice. Samples were taken before the first post-emergence application, after the first post-emergence application, after the second post-emergence application, and once the post-emergence applications were finished. The first and second post-emergence applications reduced the average density by 41% and 12%, respectively, throughout the department. Between the first and fourth evaluations, the density of the weeds and crops decreased throughout the department by 51.7% and 39%, respectively.

Rishi Raj*et al*.(2016) conducted a field experiment to evaluate suitable herbicide options for controlling weeds in transplanted aromatic rice *(Oryza sativa L.).*The experiment result was Single application of ethoxysulfuron (PoE) significantly reduced the population of small flower umbrella sedge (*Cyperusdifformis L.)* at all growth stages of rice as compared to the other herbicide used. Among the herbicide treatments, significant higher weed-control efficiency (WCE) was recorded with the sequential application of pendamethalin (PE) fbbispyribac-sodium (PoE) to the tune 92.4 and 88.2%, respectively as compared to weedy check at 30 and 90 DAT of crop. Minimum total weed dry biomass (21.1 g/m^2) and highest weed-control efficiency (84.8%) were also recorded with application of pendimethalin (PE) fbbispyribac-sodium (PoE). Sequential application of pre-and post-emergence herbicide had significant effect on yield and yield attributes of aromatic rice as compared to single application of pre- or post-emergence herbicides.

GulshanMahajan and Bhagirath S. Chauhan (2013) carried out an experiment to find outsuitable herbicide options for controlling weed in dry-seeded aromatic rice. Results indicated that the single application of pendamethalin (750 g ai/ha) PRE, pyrazosulfuron (15 g ai/ha) PRE, bispyribac-sodium (25 g ai/ha) POST, penoxsulam (25 g ai/ha) POST, and azimsulfuron (20 g ai ha-1) POST reduced total weed biomass by 75, 68, 73, 70, and 72%, respectively, compared with the non treated control at flowering stage of the crop. Grain yield following the single application of pendamethalin PRE, pyrazosulfuron PRE, bispyribac-sodium POST, penoxslum POST, and azimsulfuron POST increased by 149, 119, 138, 124, and 144%, respectively. The sequential application of herbicides proved better than single applications. The lowest weed biomass was observed with the sequential application of pendamethalin PRE followed by azimsulfuron POST, and rice yielded 228% more than the non treated control following this treatment.

Jie Chen *et al*. (2021) carried out an experiment to study the effects of herbicides on microbial community and urease activity in the rhizosphere soil of maize is helpful to clarify the mechanisms herbicides used to affect soil microbial environment. The result revealed that the bacterial community and urease activity in the 0-20 cm rhizosphere soil are suitable indices to evaluate the effects of pre emergence herbicides on maize growth and soil microbial environment.

ShashankTyagi *et al*. (2018) conducted a field experiment during rabi season to study the effect of different weed control practices on soil microbial population dynamics at various time intervals of winter maize. Results indicated that the effect of herbicides on soil microbes is only temporary. The adverse effects of herbicides were gradually reduced with passage of time and practically, there was no adverse effect of acetachlor, 2, 4-Diethyl ester and atrazine herbicides on soil microbial activities in terms of fungi, bacteria and actinomycetes population after harvest of maize

Effects of the use of the herbicide pendamethalin, hence forth referred to as PM, on green pea plants at pre-emergence and post-emergence were studied by Nahla Salim Hammok and Fathi Abdullah Al-mandee (2020). Different concentrations of the herbicide were applied at 0.0, 1.4, 2.8 and 4.1ml/l, levels which were equal to doses in the field of 0.5, 1, 1.5 l/Donum. Post-emergent plants showed a significant decrease in plant height (cm), pod number/plant, pod weight (g), pod length (cm), fresh weight of plant (g) and weight of 1000 seeds (g). However, the decrease was not significant in the dry weight of plant (g) and the number of seeds/pod. Also, all studied traits decreased significantly when different concentrations of PM overlapped with the application methods, except in the case of the weight of 1000 seeds. These results showed that the effects of PM reps are apparent from low concentrations of the herbicide, and also that treatment at post emergence reduces environmental pollution and protects the crop.

V.R. Sathya *et al* (2018) conducted an experiment to examine the effect of two pre-emergence herbicides (pendamethalin and alachlor) singly and together with two post-emergence herbicides (imazethapyr and fenoxaprop-p-ethyl) on the microbial activities of soils, cropped with black gram. Application of pendamethalin and alachlor alone or together with imazethapyr has no deleterious effect on soil microorganisms and their activities. The herbicide fenoxaprop-p-ethyl when applied together with either pendamethalin or alachlor significantly reduced the fungal population in the root zone soil including including spore numbers and the colonization of mycorrhizal fungi of black gram roots.

P. Adhikary *et al*. (2014) conducted a field experiment to assess the impact of three commonly used herbicides (pendamethalin, oxyfluorfen and propaquizafop) on soil microbial populations in chilli. Their study showed that the herbicide treatments significantly inhibited the development of microbial populations in the soil, and the degree of inhibition varied with the types of herbicide. Increasing trend of inhibition on growth of microbial populations was observed from the initial effect until 15 DAA. No inhibition was observed at 15 DAA to harvest. The study suggests that the herbicide application to soil cause transient impacts on microbial population growth, when applied at recommended field application rate.

P.C.Latha and H.Gopal (2010) conducted an experiment to assess the effect of the herbicides viz., 2,4-DEE, butachlor, pretilachlor and pyrazosulfuron ethyl at different concentrations on total heterotrophic bacteria, fungi and actinomycetes in rice field. The results of this experiment revealed that the application of herbicides reduced the population of all the bacteria counted during the study with butachlor showing highest reduction in the populations. This effect was stronger with increasing concentration of the herbicides employed.

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Chapter III

Materials and Methods

CHAPTER III

MATERIALS AND METHODS

The experiment was conducted in Agronomy research field and microbiology laboratory at BRRI Gazipur during the period from July 2020 to November 2020. This chapter deals with a brief description on experimental site, climate, soil, land preparation, layout of the experimental design, intercultural operations, data recording and their analyses.

3.1. Experimental details

3.2. Site description

The research work was carried out at the experimental field/Agronomy research field and microbiology laboratory, BRRI, Gazipur. The soil of the experimental plots belonged to the Agro Ecological Zone Madhupur Tract (AEZ-28).

3.3. Geographical location

The experimental area was situated at the latitude is 23.989014, and the longitude is 90.418167. BRRI, Gajipur, Bangladesh is located at Bangladesh category with the gps coordinates of 23° 59' 20.4504" N and 90° 25' 5.4012" E.at an altitude of 8 meter above sea level.

3.4. Agro-ecological region

The experimental field belongs to the Agro-ecological zone of “The Modhupur Tract”, AEZ-28 (Anon., 1988a). This was a region of complex relief and soils developed over the

Modhupur clay, where floodplain sediments buried the dissected edges of the Modhupur Tract leaving small hillocks of red soils as 'islands' surrounded by floodplain (Anon., 1988b).

3.5. Soil and Climate of Experimental Site:

Bangladesh Rice Research Institute, Joydebpur,Gazipur is medium high land with fine-textured (clay loam) terrace soils. It belongs to Chhiata series (Soil taxonomy: UdicRhodustalf) under the agroecological zone - Madhupur Tract (AEZ-28). Top soils were clay loam in texture, olive-gray with common fine to medium distinct dark yellowish brown mottles. The pH value ranges from 5.5 to 6.0 in the topsoil and had organic matter content is low. The experimental area was flat having available irrigation and drainage system. The land was above flood level and sufficient sunshine was available during the experimental period. Soil samples from 0-60 cm depths were collected from experimental field. The chemical analyses were done in the laboratory of the BRRI farm. The area received average rainfall 393.53 mm during July to November. The mean air temperatures during July to November of the experiment was 31°C. The average humidity (%) 78 during July to November.

3.6. Crop/Planting material

BRRI dhan75 was used as the test crop. The variety BRRIdhan75 was developed by Bangladesh Rice Research Institute (BRRI), Joydebpur, Gajipur, Bangladesh for Aman season. Main characteristics of this rice is high yielding variety, plant height 101-110 cm, stem stout, not lodging, leaf deep green, grain golden color, slight aromatic, 1000 grain weight 21 g. Life time is 110-115 days.

3.7. Soil Collection:

Soil samples (0-60 cm) were collected from herbicide treated plot at 3, 7, 10, 20, 30 and 60 days after herbicide application (DAHA) for determination of soil microorganisms.

3.8. Experimental Treatments:

The single factor experiment was compared with four treatments of herbicides**:**

T_1=Pre-emergence, Pendamethalin(33EC)

T_2=Post-emergence, Phenoxlum(20EC)

T_3=Late post-emergence,Ethoxysulfuron

T_4= Control (no herbicide).

All other fertilizers were applied in every treatment @BRRI Recommended dose.

3.9. Herbicide used in this experiment:

Pre-emergence, Pendamethalin (33EC)=234ml/bigha, Post-emergence, Phenoxlum (20EC)=12.5ml/bigha, Ethoxysulfuron=14g/bigha.

3.10. Characteristics of each herbicide:

Pendamethalin is a selective pre-emergence herbicide of dinitroaniline group extensively used for weed control. It inhibits cell division and cell elongation. Phenoxy herbicide (Phenoxlum) is selective post-emergence herbicide that induce similar physiological responses to endogenous plant hormones, such as the auxins, gibberellins, cytokinins, abscisic acid, and ethylene, and may act as competitors or antagonists of these natural plant growth regulators.

Ethoxysulfuron 15% WDG. Sunrice is a post-emergent broad spectrum herbicide very effective for the control of sedges and broad leaf weeds in transplanted rice. Sunrice contains Ethoxysulfuron as active ingredient which belongs to sulfonyl urea group of herbicides. Ethoxysulfuron is mainly taken up by the leaves and is translocated within the plant. After inhibition of plant growth, chlorotic patches develop and spread at first acropetally, then basipetally. The action of the product reaches its conclusion about 3-4 weeks after application with the death of the whole plant.

3.11. Experimental Design:

The experiments were conducted with Randomized Complete Block Design (RCBD) with three replications in field condition and Completely Randomized Design (CRD) in laboratory condition. The unit plot size was 9.6 squaremetre.Urea, TSP and MOP were applied following recommended fertilizer rate. No weeding was done in control plot.

3.12. Soil microorganisms counting methods

Nutrient Agar Media were used for total bacteria count. Fungal population was determined using Potato Dextrose Agar (PDA) Media. Nitrogen fixing bacterial (NFB) media was prepared by using Malic acid (5g), K2HPO4 (0.5g) MgSO4. 7H2O (0.2), NaCl (0.1g), CaCl2 (0.02 g), Free Media (Prasad et al., 2007) was used for free living nitrogen. Phosphate Solubilizing Bacteria (PSB) population was determined using NBRIP media plates.

The total up of the colonies was used to calculate the colony-forming unit (CFU)/g dry weight of soil using the formula: CFU/g dry weight of soil = Colony-forming unit * dilution factor/Amount of a aliquot *dry weight of soil (g).

3.13. Data analysis technique

The collected data were compiled and analyzed statistically using the analysis of variance (ANOVA) technique with the help of a computer package program name Statistix10. The significance of the difference among the treatment means was estimated by the Duncan's Multiple Range Test (DMRT) at 5% level of probability (Gomez and Gomez, 1984).

Chapter IV
Results and Discussion

CHAPTER IV

RESULT AND DISCUSSION

This chapter comprises of the presentation and discussion of the results obtained due to the effects of herbicides on microbial community in the soil of rice (BRRIdhan75) is helpful to clarify the mechanisms herbicides used to affect soil microbial environment. The results of the present investigation have been presented, discussed and compared as far as available with the results of the researchers.

4.1. Effect of herbicides on Total Bacteria (NA) in T. Aman field

Numbers of microorganisms in rice cultivating soil were initially reduced with the application of these herbicides but their numbers could be recovered 10-30 days after the applications. Incase of Pendamethalin 33EC total bacteria (Bacillus, Agrobacterium, Pseudomonas, Arthobacter etc.) could be recovered 30-60 days after its application. In case of Phenoxlum 20EC total bacteria could be recovered their numbers 10 days after its application. . In case of Ethoxysulfuron, total bacteria could be recovered their numbers 20 days after its application. Increasing trend of total bacteria were found in control plots from 0 day to 60 days.

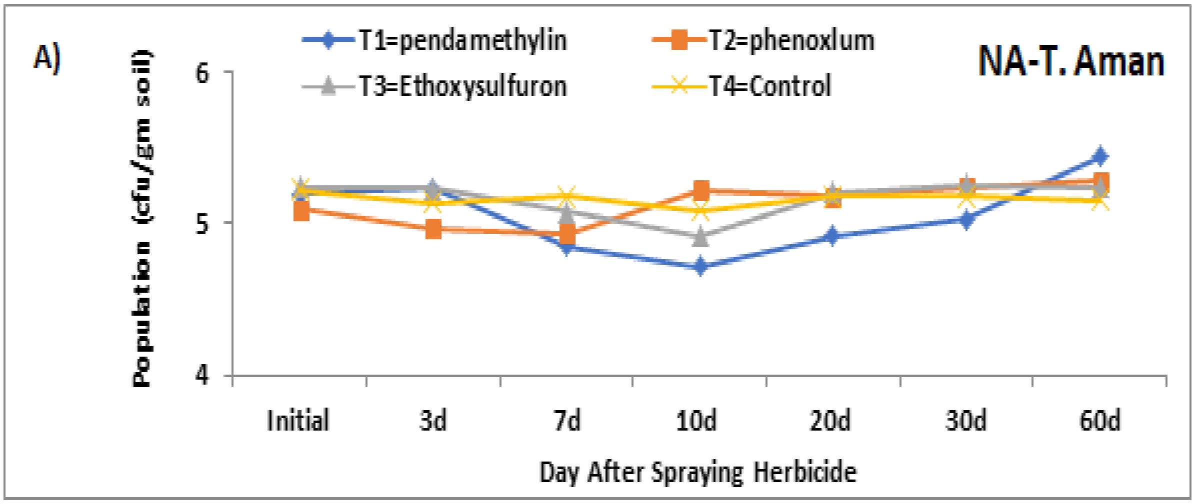

Figure 1 (A): Effect of spraying herbicides on Total Bacteria in T. Aman field (NA)

4.2. Effect of herbicides on Nitrogen fixing Bacteria (NFB) in T. Aman field

Numbers of microorganisms in rice cultivating soil were initially reduced with the application of these herbicides but their numbers could be recovered 10-30 days after the applications. In case of Pendamethalin 33EC, NFB (Azotobacter, Rhizobium, Azospirillum,Cyanobacteria etc.) could be recovered 20 days after its application. In case of Phenoxlum 20EC, NFB could be recovered their numbers 10 days after its application. . In case of Ethoxysulfuron, NFB could be recovered their numbers 20 days after its application. Increasing trend of NFB were found in control plots from 0 day to 60 days.

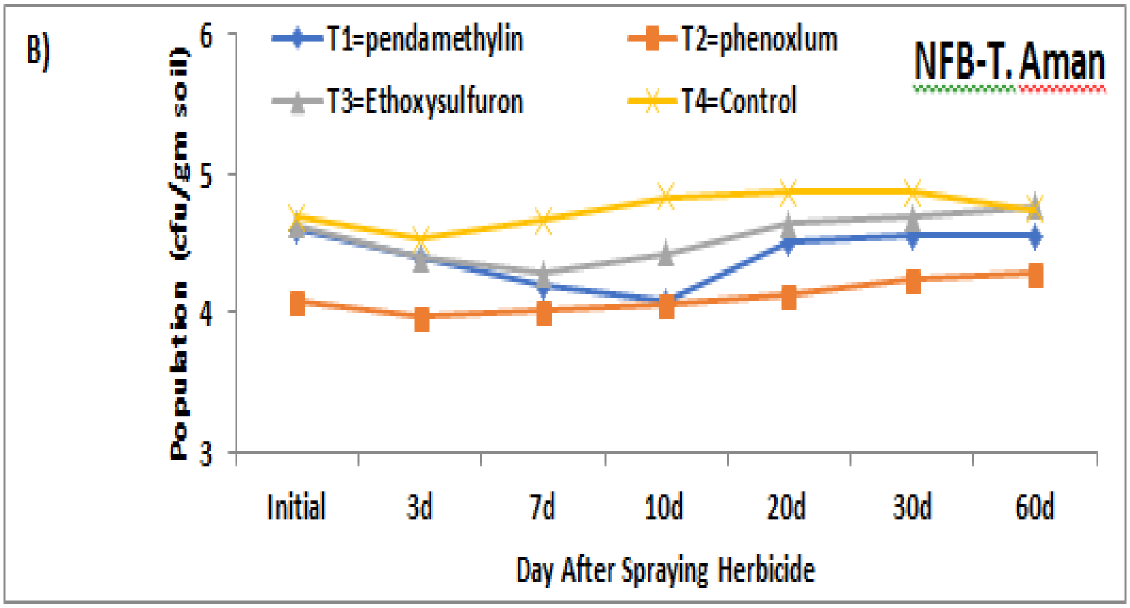

Figure 2 (B): Effect of spraying herbicides on NFB in T. Aman field

4.3. Effect of herbicides on Phosphate Solubilizing Bacteria (PSB) in T. Aman field

Numbers of microorganisms in rice cultivating soil were initially reduced with the application of these herbicides but their numbers could be recovered 10-30 days after the applications. In case of Pendamethalin 33 EC PSB (Pseudomonas, Bacillus, Fusarium, Aspergillus etc.) could be recovered 20 days after its application. In case of Phenoxlum 20EC PSB could be recovered their numbers 20 days after its application. . In case of Ethoxysulfuron, PSB could be recovered their numbers 10 days after its application. Increasing trend of PSB were found in control plots from 0 day to 60 days.

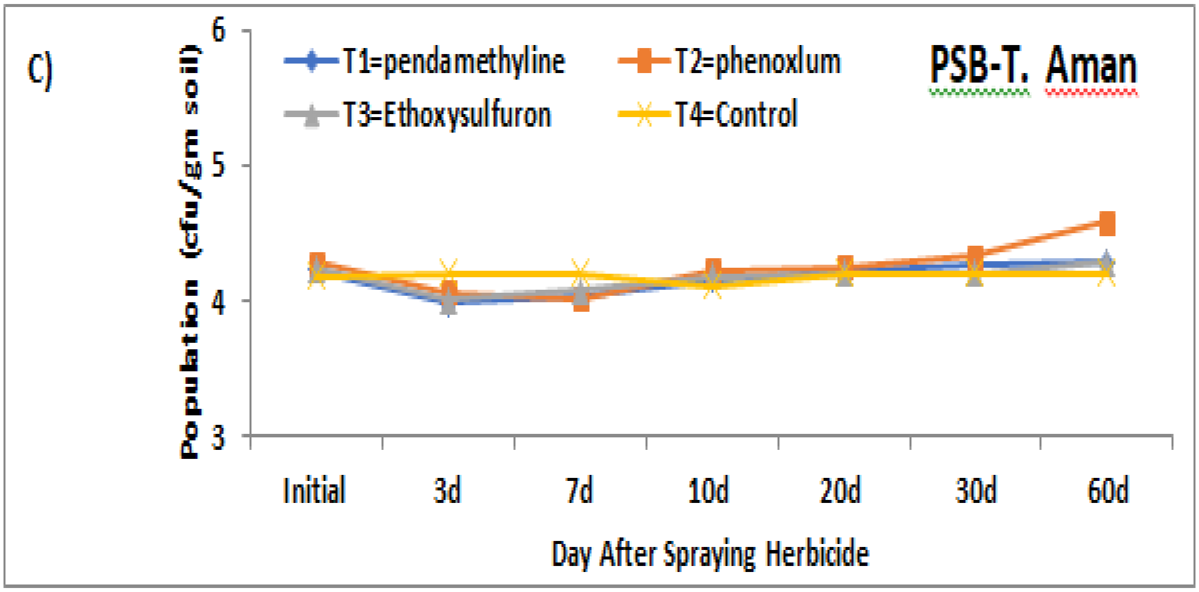

Figure 3 (C): Effect of spraying herbicides on PSB in T. Aman field

4.4. Effect of herbicides on fungus in T. Aman field (PDA)

Numbers of microorganisms in rice cultivating soil were initially reduced with the application of these herbicides but their numbers could be recovered 10-30 days after the applications. In case of Pendamethalin 33EC, Fungus (Verticillium, Phytophthora, Rhizoctonia and Pythium) could be recovered their numbers 7 days after its application. In case of Phenoxlum 20EC, Fungus could be recovered their numbers 20 days after its application. . In case of Ethoxysulfuron, Fungus could be recovered their numbers 20 days after its application. Increasing trend of Fungus were found in control plots from 0 day to 60 days.

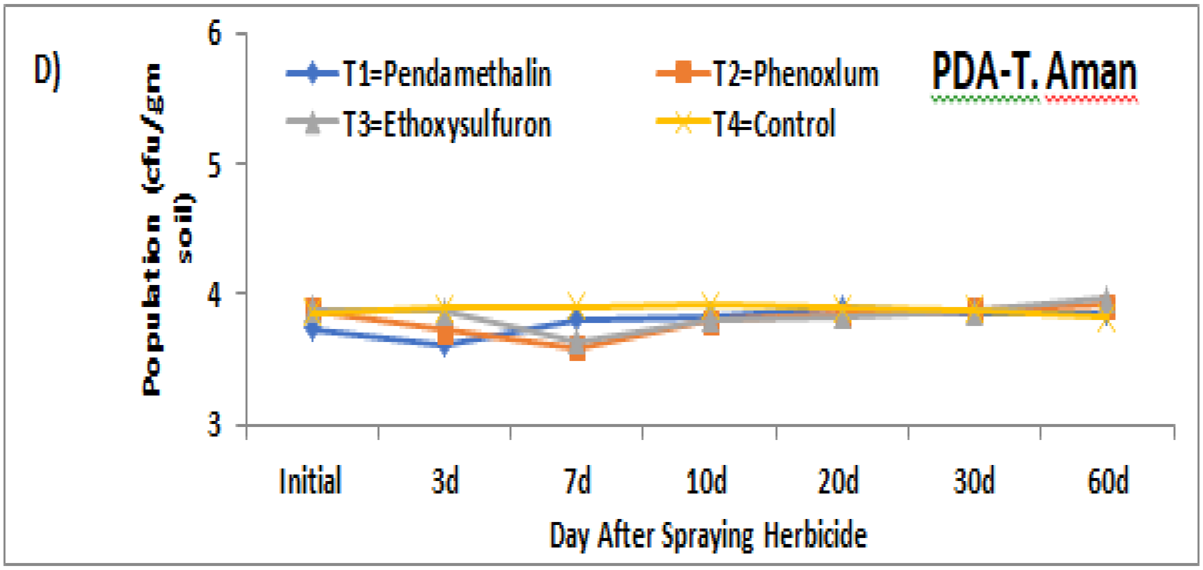

Figure 4 (D): Effect of Spraying herbicides on fungus in T.Aman field (PDA)

4.5. Total Bacteria (%) (NA):

Figure 5 shows the different herbicide treatments on total bacteria in soil. In case of Pendamethalin 33 EC (Fig 5 A) total bacteria (%) were initially reduced at 3 to 10 days but gradually total bacteria (%) could be recovered at 30-60 days after its application. In case of Phenoxlum 20EC (Fig 5 B) total bacteria (%) were initially reduced at 3 to 7 days but gradually total bacteria (%) could be recovered their numbers 10 days after its application. In case of Ethoxysulfuron (Fig 5 C) total bacteria (%) were initially reduced at 3 to 10 days but gradually but total bacteria(%)could be recovered their numbers 20 days after its application. Incase of control plots (Fig 5 D) total bacteria (%) were found in from 0 day to 60 days.

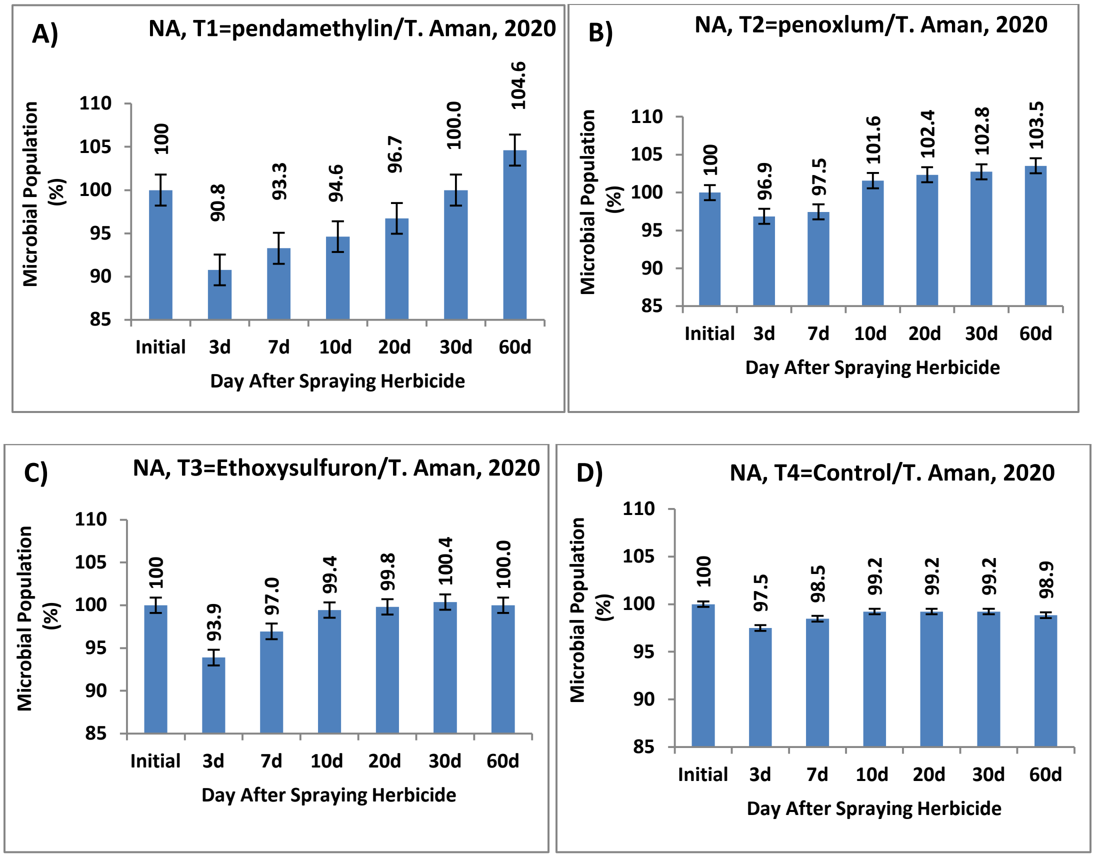

Figure 5 (A): Effect of pendamethylin herbicides on total bacteria in soil ; B) Effect of penoxlum herbicides on total bacteria in soil ; C) Effect of Ethoxysulfuron herbicides on total bacteria in soil ; D) Effect of no spraying herbicides on total bacteria in soil, T. Aman 2020

4.6. Nitrogen Fixing Bacteria (%) (NFB):

Figure 6 shows the different herbicide treatments on Nitrogen Fixing Bacteria in soil. In case of Pendamethalin 33 EC (Fig 6 A) Nitrogen Fixing Bacteria (%) were initially reduced at 3 to 10 days but gradually Nitrogen Fixing Bacteria (%) could be recovered at 20 days after its application. In case of Phenoxlum 20EC (Fig 6 B) Nitrogen Fixing Bacteria (%) were initially reduced at 3 to 7 days but gradually Nitrogen Fixing Bacteria (%) could be recovered their numbers 10 days after its application. In case of Ethoxysulfuron (Fig 6 C) Nitrogen Fixing Bacteria (%) were initially reduced at 3 to 10 days but gradually but Nitrogen Fixing Bacteria (%) could be recovered their numbers 20 days after its application. In case of control plots (Fig 6 D) Nitrogen Fixing Bacteria (%) were found in from 0 day to 60 days.

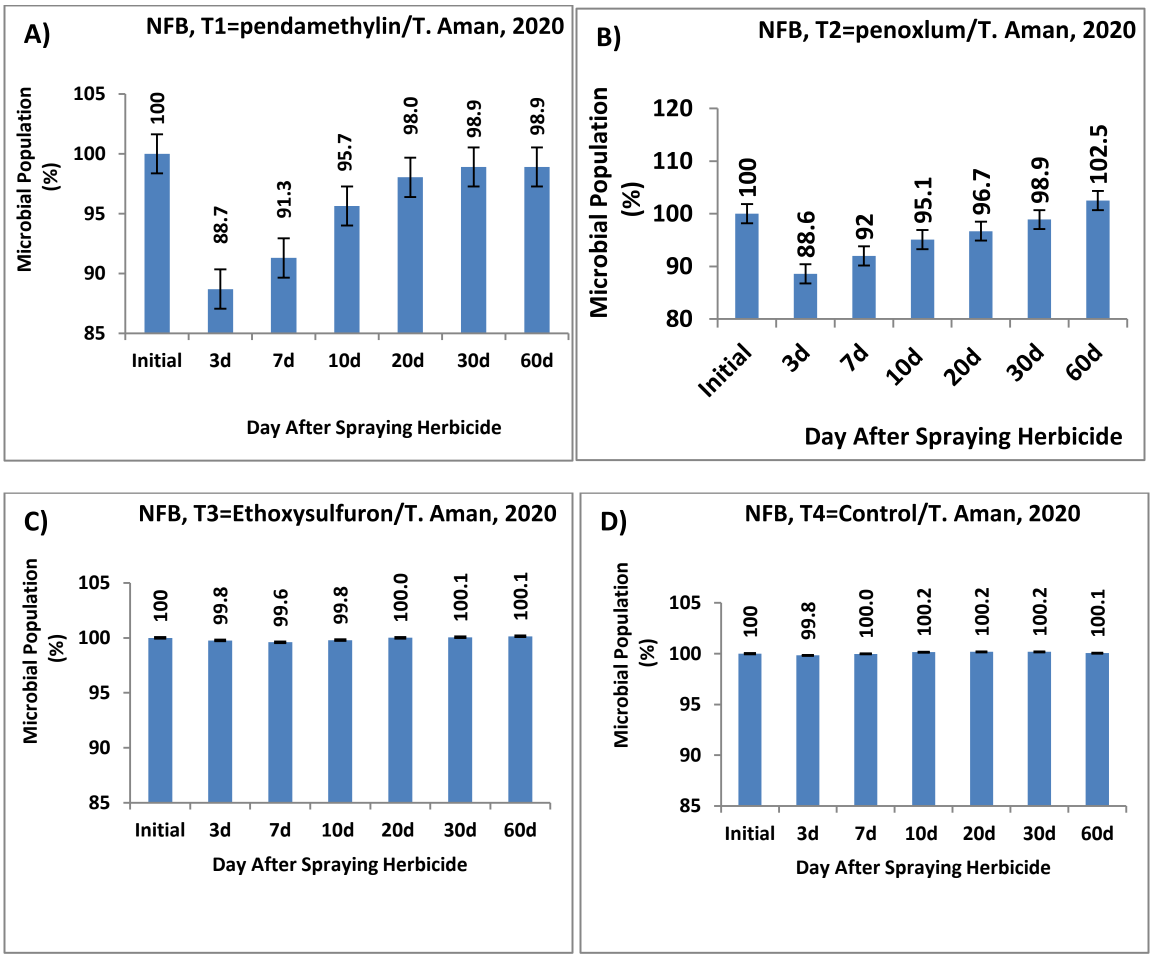

Figure 6 (A): Effect of pendamethylin herbicides on Nitrogen Fixing Bacteria (NFB) in soil ; B) Effect of penoxlum herbicides on Nitrogen Fixing Bacteria (NFB) in soil ; C) Effect of Ethoxysulfuron herbicides on Nitrogen Fixing Bacteria (NFB) in soil ; D) Effect of no spraying herbicides on Nitrogen Fixing Bacteria (NFB) in soil, T. Aman 2020.

4.7. Phosphate Solubilizing Bacteria (%) (PSB):

Figure 7 shows the different herbicide treatments on Phosphate Solubilizing Bacteria in soil. In case of Pendamethalin 33 EC (Fig 7 A) Phosphate Solubilizing Bacteria (%) were initially reduced at 3 to 10 days but gradually Phosphate Solubilizing Bacteria (%) could be recovered at 20 days after its application. In case of Phenoxlum 20EC (Fig 7 B) Phosphate Solubilizing Bacteria (%) were initially reduced at 3 to 10 days but gradually Phosphate Solubilizing Bacteria (%) could be recovered their numbers 20 days after its application. In case of Ethoxysulfuron (Fig 7 C) Phosphate Solubilizing Bacteria (%) were initially reduced at 3 to 10 days but gradually Phosphate Solubilizing Bacteria (%) could be recovered their numbers 20 days after its application. Incase of control plots (Fig 7 D) Phosphate Solubilizing Bacteria (%) were found in from 0 day to 60 days.

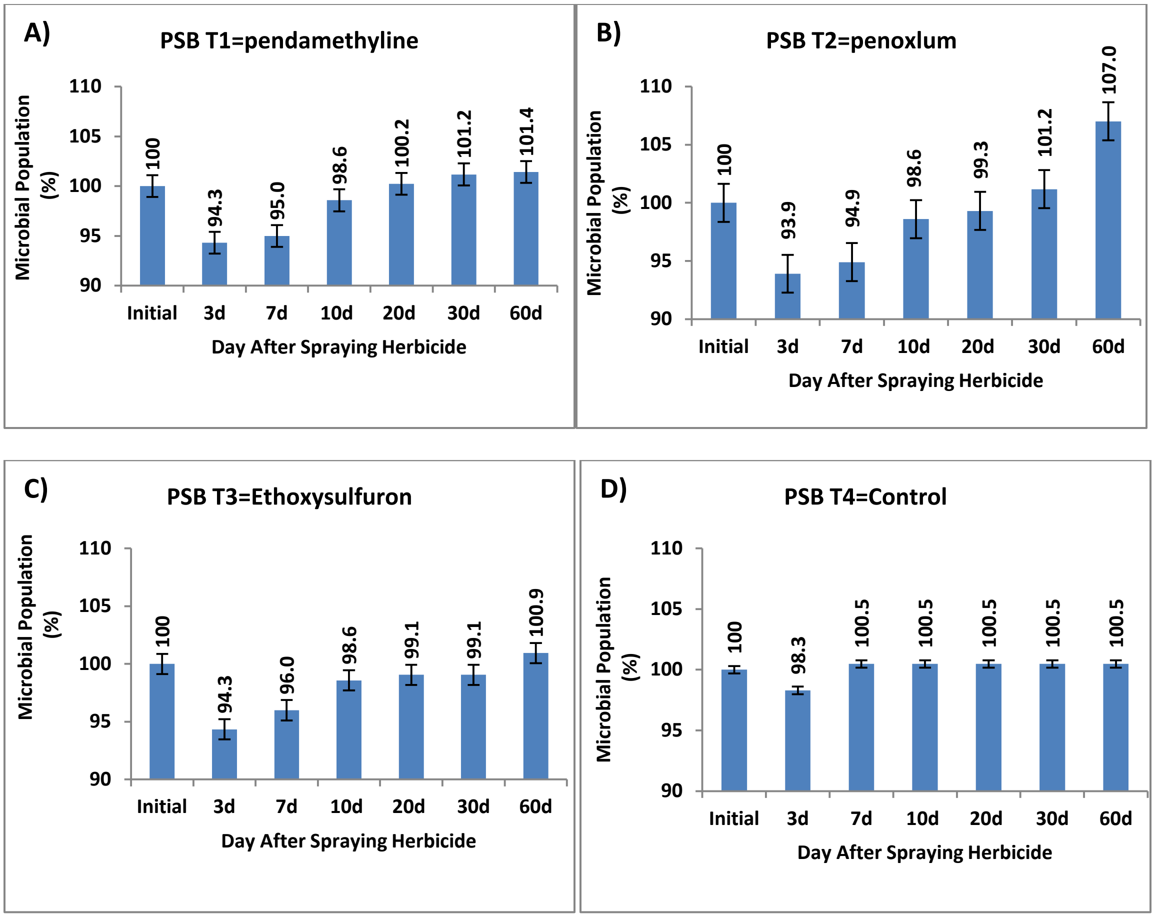

Figure 7 (A): Effect of pendamethylin herbicides on Phosphate Solubilizing Bacteria (PSB)in soil ; B) Effect of penoxlum herbicides on Phosphate Solubilizing Bacteria (PSB) in soil ; C) Effect of Ethoxysulfuron herbicides on Phosphate Solubilizing Bacteria (PSB)) in soil ; D) Effect of no spraying herbicides on Phosphate Solubilizing Bacteria (PSB) in soil, T. Aman 2020.

4.8. Fungus (%):

Figure 8 shows the different herbicide treatments on total bacteria in soil. In case of Pendamethalin 33EC (Fig 8 A) Fungus (%) were initially reduced at 3 days but gradually Fungus (%) could be recovered at 7 days after its application. In case of Phenoxlum 20EC (Fig 8 B) fungus (%) were initially reduced at 3 to 10 days but gradually Fungus (%) could be recovered their numbers 20 days after its application. In case of Ethoxysulfuron (Fig 8 C) Fungus (%) were initially reduced at 3 to 10 days but gradually but fungus (%) could be recovered their numbers 20 days after its application. Incase of control plots (Fig 8 D) Fungus (%) were found in from 0 day to 60 days.

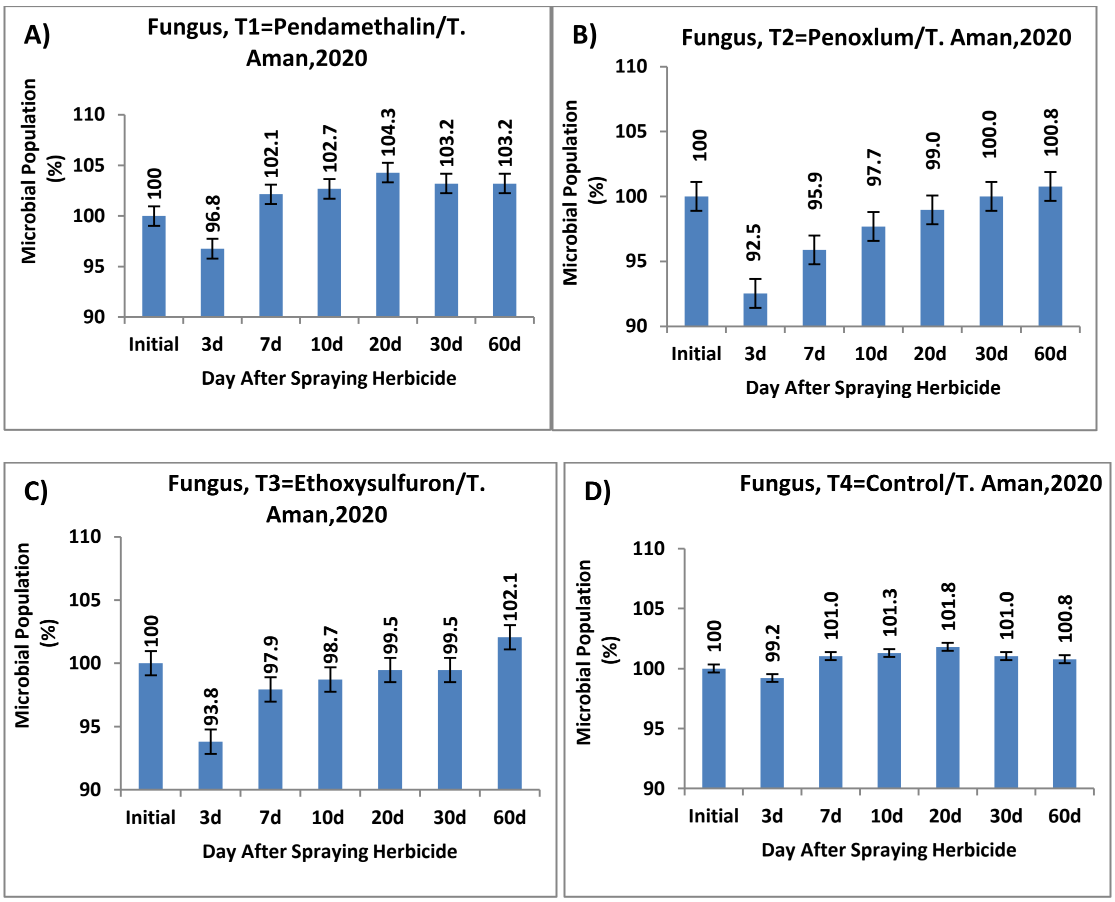

Figure 8 (A) Effect of pendamethylin herbicides on fungus in soil ; B) Effect of penoxlum herbicides on fungus in soil ; C) Effect of Ethoxysulfuron herbicides on fungus in soil ; D) Effect of no spraying herbicides on fungus in soil, T. Aman 2020.

4.9. Panicles/m^2

Table 2 shows the effects of different treatments on panicles/m2. Panicles/m^2 was significantly influenced by the herbicide treatments. Panicles/m^2 was increased due to the herbicides treatment compare to the control treatment. The highest panicle/m^2 (200.33) was obtained in the T1 (Pendamethalin 33EC) treatment which was significantly greater than T4 control (No herbicide) treatment. The lowest panicle/m^2 was obtained in the T4 (control) treatment. In terms of panicles/m^2 the treatments may be arranged as T1>T2>T3>T4**.**

Table: 1. Effect of herbicides on yield contributing character (panicle/m^2) of BRRIdhan75

Treatments	**Panicles/m^2**	**Percent increase over control**
T_1=Pendamethalin (33EC)	200.33a	43.78
T_2= Phenoxlum (20EC)	171.33b	22.97
T_3= Ethoxysulfuron	169b	21.29
T_4= Control (no herbicide).	139.33c	------
CV	6.41	------
LSD (0.05%)	21.772	------

4.10. Grains/panicle

Table 3 shows the effects of different treatments on grains/panicle. Grain/panicle was significantly influenced by the herbicide treatments. Grains/panicle was increased due to the herbicides treatment compare to the control treatment. The highest grain/panicle (133.34) was obtained in the T1 (Pendamethalin 33EC) treatment which was significantly greater than T4 control (No herbicide) treatment. The lowest grain/panicle was obtained in the T4 (control) treatment. In terms of grain/panicle the treatments may be arranged as T1>T2>T3>T4**.**

Table: 2. Effect of herbicides on yield contributing character (Grains/panicle) of BRRIdhan75

Treatments	**Grains/panicle**	**Percent increase over control**
T_1=Pendamethalin (33 EC)	116.67a	133.34
T_2= Phenoxlum (20 EC)	74.33b	48.66
T_3= Ethoxysulfuron	66c	32
T_4= Control (no herbicide).	50 d	------
CV	3.36	------
LSD (0.05%)	5.1478	------

4.11. Grain yield (t/ha)

Table 1 shows the effects of different treatments on grain yield. Grain yield was significantly influenced by the herbicide treatments. Grain yield was increased due to the herbicides treatment compare to the control treatment. The highest grain yield (4.35 t/ha) was obtained in the T1 (Pendamethalin 33EC) treatment which was significantly greater than T4 control (No herbicide) treatment. The lowest grain yield (3.1 t/ha) was obtained in the T4(control) treatment. In terms of grain yield the treatments may be arranged as T1>T2>T3>T4.

Table: 3. Effect of herbicides on yield of BRRIdhan75

Treatments	**Yield(t/ha)**	**Percent increase over control**
T_1=Pendamethalin (33EC)	4.35a	40.32
T_2= Phenoxlum (20EC)	4.15ab	33.87
T_3= Ethoxysulfuron	3.78b	21.94
T_4= Control (no herbicide).	3.1c	------
CV	5.43	------
LSD (0.05%)	0.4169	------

Chapter V

Summary and Conclusion

CHAPTER V

SUMMARY AND CONCLUSION

Herbicides being biologically active compounds, an unintended consequence of the application of herbicides is that it may lead to significant changes in the populations of microorganisms and their activities thereby influencing the microbial ecological balance in the soil and affecting the productivity of soils. Weed management is challenging task in agriculture to maintain crop yield and productivity. This fact is also true for aromatic fine rice, as it is one of the factors responsible for lowering the grain yield. However, very little research has been conducted in aromatic fine rice of Bangladesh as well as their weed management. The increasing reliance of rice cultivation on herbicides has led to concern about their Eco-toxicological behavior in the rice field environment.

In the current research it was observed that numbers of microorganisms in rice cultivating soil were initially reduced with the application of these herbicides but their numbers could be recovered 10-30 days after the applications. In case of Pendamethalin 33EC, fungus could be recovered their numbers 7 days after its application, NFB & PSB could be recovered 20 days after its application and total bacteria could be recovered 30-60 days after its application. In case of Phenoxlum 20EC, NFB & total bacteria could be recovered their numbers 10 days after its application whereas PBS & fungus could be recovered their numbers 20 days after its application. In case of Ethoxysulfuron, NFB, total bacteria & fungus could be recovered their numbers 20 days after its application whereas PBS could be recovered their numbers 10 days after its application. Increasing trend of total nitrogen, nitrogen fixing bacteria, phosphate solubilizing bacteria and fungus were found in control plots from 0 day to 60 days. The highest yield (4.35 t/ha) was obtained from Pendamethalin 33EC treated plots in BRRIdhan75 compared to control .

However, this experiment was conducted only in one location and one year. Multi-location trials for several years should be conducted before recommending the package to the farmers. Moreover, environmental issues related to herbicide use should be brought under consideration. Because, farmers sometimes ignore the instructions and do not use the correct volume of spray, dose and spray nozzle, which lead to poor weed suppression. In that case farmers may integrate pre– or post–emergence herbicide with a manual weeding considering both the environmental and labour cost issues.

Recommendations

From the above experimental findings, it is apparent that the application of all herbicides shifts soil microbial community structure that are not harmful for transplanted rice and performed better on yield and yield parameters of BRRI dhan75. So farmer would be used of these three herbicides in field condition to control weed.

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