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ORIGINAL ARTICLE

Relationship between nitrogen fertilization and rice grain (*Oryza sativa* L.) properties

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ABSTRACT
I*n order to evaluation of the effect of chemical and biological fertilizer on yield , yield components, nitrogen uptake and quality parameters of rice , one variety (Hashemi) was investigated in an experiment as factorial in RCBD with 3 replications in a paddy light soil at Guilan province, Iran, (2008-2009). In this experiment 4 biological treatments including:M1-control (no fertilizer) ; M_2 -10 ton/ha cow dung ; M_3-20 ton/ha cow dung and M_4 -5 ton/ha azolla compost , and 4 chemical treatments including: S_1-control (no N fertilizer); S_2-40 kg N /ha; S_3-60 kg N /ha and S4-80 kg N /ha were compared. Results showed that:in Biological fertilization M4 produced maximum grain yield (3387kg/ha) also in chemical fertilizers by increasing of N rate, yield increased, as maximum yield was observed in S_4 (3373kg/ha).Also interaction effect of N application on yield and biomass increasing was remarkable too, whereas M4S4 created the highest yield and biomass (3867 and 8653 kg/ha) respectively. In addition, Study of interactions showed the most and the least grain nitrogen uptake in M_4S_3 (62.67 kgN/ha), and M_1S_1 (30.33 kgN/ha) is obtained respectively. Agricultural properties showed main panicle height (26.2 cm) and 1000 grain weight (75.5 gr) were reached the highest value by cow dung fertilization. In considering the chemical and biological fertilizers no significant difference was observed in AC whereas Interaction between chemical and biological fertilizers on AC showed , AC was the most and least in M1S1 and M4S3 (23/2 and 22/55%).Also in biological fertilization the highest protein content (9.39%) belonged to M3 whiles S3 created maximum in chemical fertilization (9.51%).*
Key Words: *Azolla, fertilizer, rice, nitrogen, protein, yield*

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INTRODUCTION

Rice provides 35- 60% of the dietary calories consumed by nearly more than 3 billion people [1]. during rice growing, nitrogen is one of the most essential nutrients for optimal yield, yield components and yield associated parameters are formed during crop growth cycle , so use of N fertilizers in chemical or biological form, adequate amount and application methods are Important management strategies of this element that affected to the agricultural properties of rice plant and can be increase grain yield parameters [2] . Recently Watanabe and Liu found, the main N loss mechanisms are volatilization of ammonia (NH_3), leaching loss of (NO_3), and loss through denitrification fertilizer application, soil conditions, volatilization and environmental factor has been suggested for increasing N efficiency [3].Increased and indiscriminate use of chemical fertilizers resulted in several harmful effects on environmental pollutions; water health also reduced the productivity of the soil by deteriorating. Soil health in terms of soil fertility and biological activity. Therefore; emphasis should be laid on reducing the use of chemical inputs and improving their use efficiency).Organic farming, refraining from the use of synthetic agrochemicals, is an option to solve these problems and thus there is a renewed interest in organic manures such as composts, farmyard manure and green manures as a source of plant nutrients [5] .Composting is a viable means of transforming various organic wastes into products that can be used safely and beneficially as bio fertilizers and soil conditioners, also the most important factor in using Azolla as a bio-fertilizer for rice crop is its decomposition in soil and availability of its nitrogen to the rice plants [6],On the other hand use of farmyard manure as a bio fertilizer not only acts as a source of N and other nutrients but also increases the efficiency of applied nitrogen, so organic matter and mixture of

chemical fertilizers determine the fertility and nutrient status of a soil [7]The present investigation was undertaken during 2008 and 2009 to have a detailed account of the effect of commercially-available nitrogenous (N) fertilizers (Urea) , Azolla compost and cow dung (as a manure) on yield and its component of commercial rice varieties in Iran. This information could be useful for increasing of yield, total biomass, agricultural properties and quality parameters of rice grain.

MATERIAL AND METHODS

A field experiment involving rice was conducted at rice institute of Rasht, Iran in 2 years (2008and 2009) .The experiment was laid out in randomized complete block design with 3 replications of 4 biological fertilizers levels (M_1: no fertilizer , M_2:10 ton /ha cow dung , M_3: 20 ton /ha cow dung and M_4: 5 ton /ha azolla compost) And 4 chemical fertilizers (S_1:no fertilizer, S_2: 40 kg N/ha , S_3:60 kg N /ha and S_4:80 kg N /ha) . Six m^2 of each plots area were harvested and dried for 2 days at 70^o C and the total biomass was recorded. Grain weight, adjusted to 14% moisture content, was used as estimates of grain yield (kg/ha).and also 3 hills in each plot were randomly selected and tagged for recording plant height , main panicle height (cm) and % whole grian . Grain nitrogen concentration were determined by the methods of micro-Kjeldal digestion, distillation, and titration [7].For determining AC color rating method was used [7]and as well as the amount of rice nitrogen with the usage of Kjeldal method was measured and with N×5.95 relation protein content (PC) was calculated [8]. MSTATC, SAS and SPSS soft wares were used in variance analysis, comparison of average and determining simple correlation between characteristics.

RESULTS AND DISCUSSIONS

Total Biomass (dry matter)

Nitrogen (N), in addition to water, is considered to be the most important input for biomass production. Based on Variance analysis, there was significant influence of year, biological and chemical fertilizer on total biomass (Table1).

Table1: total biomass (kg/ha) of rice across biological and chemical fertilization

	1st year	2nd year	Average
Biological fertilizer (M)			
No fertilizer	7436 B	5904 C	6670 B
10 ton/ha cow dung	7736 B	6820 B	7278 AB
20 ton/ha cow dung	8786 A	6340 B	7563 A
5 ton/ha azolla compost	7474 B	7408 A	7441 AB
Chemical fertilizer (S)			
No fertilizer	6468 C	5342 C	5905 C
40 kgN /ha	7571 B	6223 B	6897 B
60 kgN /ha	A 8421	7073 A	7747 A
80 kgN /ha	A 8971	7835 A	8403 A
F-test			
Year(Y)		**	
Biological fertilizer (M)		*	
Chemical fertilizer (S)		**	
Y*M		**	
Y*S		ns	
M*S		ns	
Y*M*S		ns	

*, **, NS Significant at the 5 and 1% probability level and non-significant, respectively.
Means followed by the same letter in the same column are not significantly different at the 5% probability level.

The data of two years showed that 20 ton/ha cow dung and 5 ton/ha azolla compost (M3 and M4 respectively) as a biological fertilizer produced maximum total biomass (8786 and 7408 kg.ha^{-1}) in the first and second years respectively. average results of biomass showed that, by increasing cow dung as a bio fertilizer (from M2 to M3), total biomass increased, so M_3 created the most total biomass (7563 kg N/ha),(table1); In addition regarding the average of 2 years , Azolla compost (M_4) correspond to efficiencies for biomass production(7441 kg/ha)too ,therefore, Azolla is efficient, cost effective and ecologically proven bio-fertilizer [13]and also among the chemical fertilizers, in the 2 years by increasing N rate from S1 to S4, biomass augmented as S4 treatment (80 kg N/ha) had the highest total biomass (8971 and 7835 kg ha^{-1}) in the first and second years respectively (Figure 1). showed that total biomass accumulation increased significantly with N fertilizer application in rice at all the growth stages of the crop[9]. Also Average results of 2 years showed, total biomass progressed by increasing chemical

fertilizer so S4 treatment (80 kg N/ha) created the highest biomass (8403 kg/ha) (table 4). Artacho et.al (2009) described that total biomass production had a significant response to N fertilization, too.Also, in his research presented that the higher biomass of chemical treated plants could be connected with the positive effect of nitrogen in some important physiological processes, these differences were statistically significant [11].Also intraction effect of chemical and biological fertilizers showed the program of biomass must be formulated based on azolla compost (5 ton/ha) and S_4 in order to get the highest total biomass (8653 kg/ha) in rice production ($R^2 = 0.93$) (Figure 1).

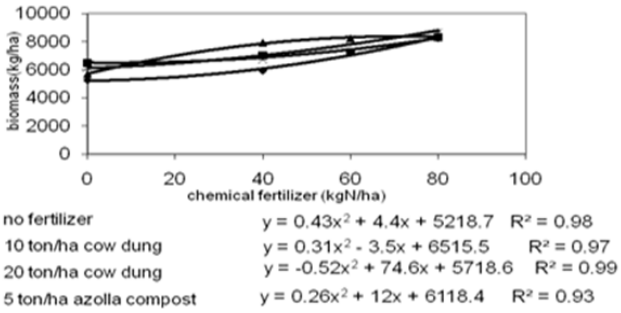

Figure1: relationship between chemical fertilizer and biomass under different biological fertilize

Grain Yield

all of data for 2 years are presented (table 2). biological fertilizer results were showed both 2 years azolla compost which produced maximum grain yield and also increasing of cow dung as a fertilizer, created positive effect on yield content, nevertheless, Maximum yield (3387kg/ha) was obtained with 5 ton/Ha azolla compost (M_4) (table 2). The effect of azolla on rice yields has been studied by several researchers. showed use of 5 to 10 ton/Ha azolla compost equivalent to 30 to 60 N kg/Ha , and Evangelista ,2001, considered that application of azlla compost to the soil improved the soil organic matter[12] . Results of chemical fertilizers showed the use of chemical fertilizers causes to increase Nitrogen rate in soil. so maximum yield was observed in S_4 (80 kgN/ha) in average results of 2 years (3373kg/ha) (table 2).Overall, by studying of average data of two years showed that grain yield ranged from 2948 to 3387 Kg/ha and 2523 to 3373 Kg/ha during biological and chemical fertilization, respectively, and in biological and chemical fertilizers azolla compost and 80 kg N /ha produced maximum yield (3387 and 3373 Kg/ha respectively) . Differences in grain yield have been widely reported and these differences are associated with the effect of application of biological and chemical fertilizers on rice. In the biological fertilization that refrain from the use of synthetic chemicals, soil microorganisms become major determinants of Nitrogen cycling and plant growth, so azolla compost as a biological fertilizers caused increasing of vegetative grow and in result increased grain yield [13].

Table 2: Grain yield (kg ha^{-1}) of rice across biological and chemical fertilizer

	1st year	2nd year	Average
Biological fertilizer (M)			
No fertilizer	2924C	2972 C	2948 B
10 ton/ha cow dung	D 2854	3050 C	2952 B
20 ton/ha cow dung	B 3081	2879 B	2980 B
5 ton/ha azola compost	A 3440	3334 A	3387 A
Chemical fertilizer (S)			
No fertilizer	D 2408	2638 D	2523 B
40 kgN /ha	C 3158	2982 C	3070 A
60 kgN /ha	B 3314	3258 B	3286 A
80 kgN /ha	A 3420	3326 A	3373 A
F-test			
Year(Y)		ns	
Biological fertilizer(M)		**	
Chemical fertilizer (S)		**	
Y*M		**	
Y*S		**	
M*S		**	
Y*M*S		**	

*, **, NS Significant at the 5 and 1% probability level and non-significant, respectively.
Means followed by the same letter in the same column are not significantly different at the 5% probability level.

Also results of this research showed Interaction between biological and chemical fertilizers caused to significant differences in the yield According to regression equations all of the biological fertilization (M1 to M4) was having quadratic responses to chemical fertilization in the range of 0 to 80 kg/ha and also Yield had a significant quadratic response to interaction N fertilization (chemical and biological), ($0.95<R^2<0.99$), so M_4S_4, created the most yield (3867kg/ha) while M_1S_1 showed the least (2320 Kg/ha) (Figure1). Indicating that, in most cases, by using of biological fertilizers and higher Nitrogen rate in chemical fertilizers would be due to higher yield(figure 2).

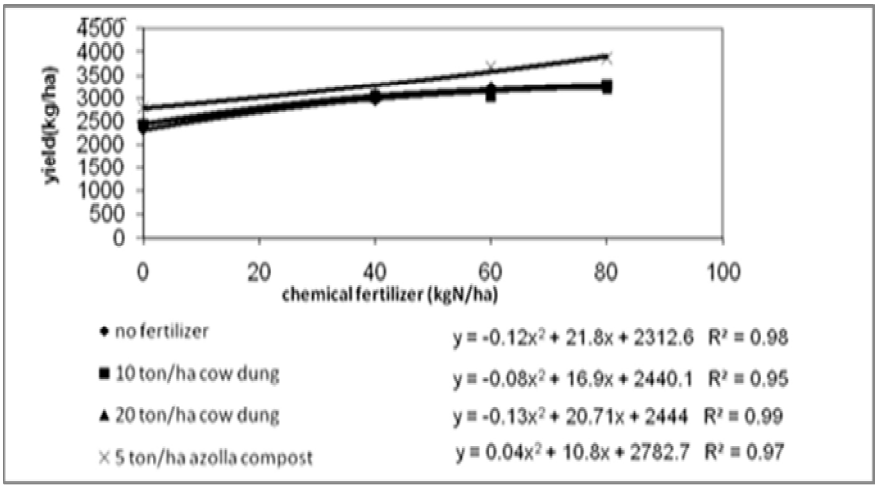

Figure 2: relationship between yield and chemical fertilizer under different biological fertilizer

Yield components

Significant differences in yield components and Nitrogen accumulation were found between years and fertilization treatments (table 3 and 4).

Plant height

In this experiment, result of variance analysis at 5% confidence level showed that chemical fertilizer had significant effect on Plant height (table 3). The findings showed that by increasing of N rate in fertilizers , plant height is increased whereas Comparing of average of data showed that maximum Plant height (135 cm) was obtained with the highest N rate (S4) (table 4). The reason for this might be due to the additional and quick supply of nitrogen facilitating the plant height [14] .Fageria et al (2003) also reported similar result as plant height increases with increasing grain yield. In addition [15] showed short height plant produces less yield and high height plant obtain more dry matter in rice that same as chemical fertilizers, result of variance analysis at 5% confidence level showed biological fertilizer had significant effect on Plant height too, as Comparing of average of data showed M3 created the highest plant (133 cm). Interaction effect of chemical and biological fertilizers had no significant on plant height.

Table 3: Significance of the F values from the analysis of variance for rice parameters

Source	df	Plant height (cm)	Main panicle height (cm)	% whole grain
Year(y)	1	6279.1**	1143.33**	0.1ns
R	4	16.6ns	0.4ns	0.4ns
M	3	59.6ns	2.1ns	37.9**
Y*M	3	129.6**	2.6ns	0.9ns
S	3	43.8*	1.8ns	3.3*
Y*S	3	118.7**	0.4ns	0.4ns
M*S	9	21.9ns	3.6ns	12.5**
Y*M*S	9	8.9ns	3.4ns	0.6ns
Cv		3.2	5.1	2.16

*, **, NS : Significant at the 1 and 5% probability level and non- significant, respectively.

Table 4: Results of comparison of average of studied variables between Fertilizers contents in confidence level of 5%

Variable	year		Biological fertilizer				Chemical fertilizer			
	2008	2009	M1	M2	M3	M4	S1	S2	S3	S4
Plant height(cm)	138a	122b	129a	130a	133a	130a	125b	130ab	132ab	135a
Main panicle height(cm)	29a	22b	26a	25.5a	26.2a	25.7a	25 a	26a	25.7a	26.1a
% whole grain	73a	73a	73b	75.5a	73b	72b	73.3ab	74ab	73b	74.7a

M1: no fertilizer, M2: 10 ton cow dung /Ha , M3: 20 ton cow dung /Ha , M4: 5ton azolla compost /Ha
S1: no fertilizer , S2: 40Nkg/ha , S3:60Nkg/ha , S4:80Nkg/ha

Main panicle height

Chemical and biological fertilization also interaction of them, had no significant effect on Main panicle height (table 3). found N fertilization more than 80 kg N/ha caused the positive effect on main panicle height but in less than this rate, the effect of fertilization is insignificant[16].

Whole grain percentage

reported that percent filled grains per panicle and total grains per panicle were the secondary or tertiary important components of yield associated with rice yield, after number of panicles/m^2[17]. Application of fertilization had significant effect on whole grain percentage (table 3) and this variable was improved with both of chemical and biological fertilization.Result of variance analysis revealed chemical and biological fertilization had significant effect on %whole grain , as the highest whole grain percentage (75.5 and 74gr) were obtained from M2 and S2 respectively. By using of chemical fertilizers whole grain percentage differs significantly between S1 to S4 (Table 4). As result of comparison of average of data showed that the highest whole grain percentage (74g) were obtained from S2 and the lowest content (73 gr) belonged to S3.Also result of variance analysis at 5% confidence level revealed that whole grain percentage had significant response to biological fertilization (Table 3) as M2 had the highest whole grain percentage (75.5) and the lowest number was due M4 (72) (table 4). Leesawatwong et al. (2005) suggested that the effect of N on decreasing breakage might have been enhanced because protein bodies occupy the space between unpacked starch granules and thus function as a binder for starch.

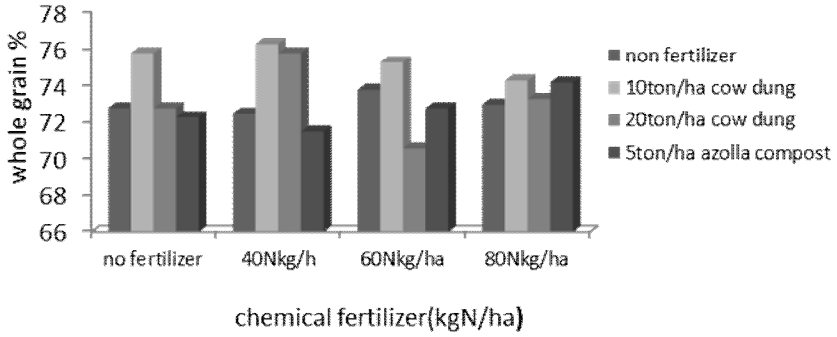

Figure 3: comparison of average of % whole grain in contraction between chemical and biological fertilizer

However, unlike the other yield component , the mutualism which existed between chemical and biological fertilizers could create a favorable impact in terms of Whole grain percentage , so the heights whole grain % belong to M2S2 (76.3).On the other hand, already had high % unbroken rice and more abundant storage protein in the lateral region with the grain of low N concentration. It is hypothesized that high density of storage protein in the lateral region of the endosperm provides resilience and lessens grain breakage during milling. The additional protein may increase hardness in rice grains and thus could make the rice more resistant to breakage during milling.

Grain nitrogen uptake

A great part of the applied N is escaped to the environment through de nitrification and volatilization [18]. Result of variance analysis at 5% confidence level revealed that nitrogen uptake in grain had a significant response to chemical and biological fertilization (Table 5) Values as averages of two years across chemical and biological fertilization showed by increasing biological application Grain nitrogen

uptake increased slightly from M_1 to M_4 and also with regard to the quantity of nitrogen fixation and nutrient recycled, Azolla compost (M_4) correspond to efficiencies for N uptake too (52 kg.ha^{-1}) (Table 6).

Table 5: Significance of the F values from the analysis of variance for ricecultivars parameters

Source	D_f	Grain N uptake(kg/ha)
Rep.	2	1.58 ns
Biological fertilizer(M)	3	**228.3
Chemical fertilize(S)	3	826**
S ×M	9	**43.83
error	30	3.38
cv		4.6

*, ** = significant at the 0.05 and 0.01 probability levels, respectively , ns = not significant

This could be due to a decrease in the differences in nutrients availability between azolla and the other biological fertilizer at growing stages as it was suggested[19]. In addition, by using of azolla reduction of the high ammonia volatilization losses in rice fields is accrued.

Table 6: Results of comparison of average of studied variables between fertilizers contents in confidence level of 5%

Variable	**Grain N uptake**
Year	
2008	47.5a
2009	47.3a
Biological fertilizer	
M1	38.5C
M2	39.9BC
M3	40.5B
M4	45.1A
Chemical fertilizer	
S1	32.5C
S2	41.2B
S3	45.4A
S4	44.9A

M1: no fertilizer, M2: 10 ton cow dung /Ha , M3: 20 ton cow dung /Ha , M4: 5ton azolla compost /Ha
S1: no fertilizer , S2: 40Nkg/ha , S3:60Nkg/ha , S4:80Nkg

Kannaiyan and Kumar (2005) were of the opinion that the most important factor in using Azolla as a bio-fertilizer for rice crop is its decomposition in soil and availability of its nitrogen to the rice plants. In addition, survey of chemical treatment showed that Grain nitrogen uptake in S_3 treatment (52 kg/ha N) was significantly higher than the other chemicals. It is obvious that all fertilizations the caused grain N uptake increases.It is important that in grain nitrogen uptake, comparing of biological and chemical fertilization showed : azolla (M4) had more positive effect than the other fertilizers specially chemical treatment. Same as these results Singh et al., 1986, Reported maximum nitrogen uptake in rice growth obtained by using azolla treatment and this treatment showed significantly more N uptake than the urea during harvest season. Hobbit's Study of interactions showed that the most and the least grain nitrogen uptake in M_4S_4 (51.2 kg ha^{-1}), (R^2 = 0.9) and M_1S_1 (27.8 kg ha^{-1}, R^2 = 0.1) are obtained, respectively (figure 4).Fageria (2003) reported that in cereals including rice, N accumulation is associated with dry matter yield of shoot and grain.Overall, nitrogen accumulation in the grain was about 51% higher compared to shoot. Distribution of N in the shoot and grain varied with the genotypes. However, across the genotypes, N accumulation of 60% in the grain and 40% in the shoot were observed.

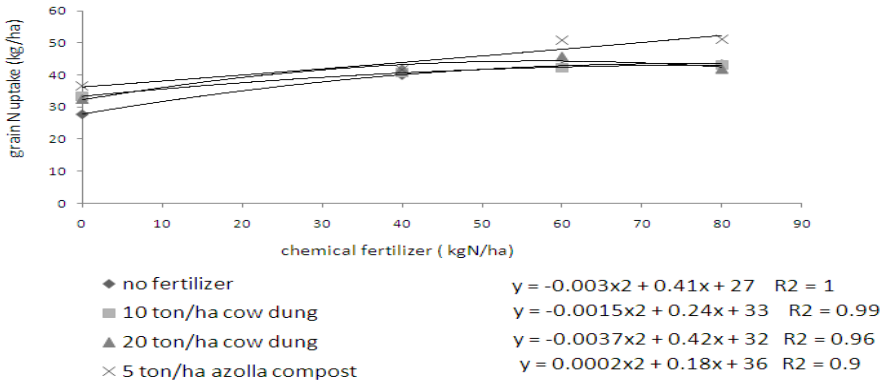

Figure 4: relationship between chemical fertilizer and grain nitrogen uptake under different biological fertilizer

Quality properties

Texture is an important attribute of food acceptance by consumers and as such, a critical step in quality assessment. Texture is defined as "the sensory manifestation of the structure of food and the manner in which that structure reacts to applied force" (Szczesnick, 1968).Rice texture is affected by factors such as rice variety and amylose content (AC) (Meullenet et al., 1998). amylose content (AC) is one of the major rice traits that is directly related to cooking and eating quality (Little et al. 1958).AC is responsible for texture and appearance in rice. Hence, regulating AC in rice has been a major concern of rice breeders. To facilitate the development of new varieties with high cooking and eating qualities, it is necessary to understand the genetic bases of such traits.

Table 7: Results of comparison of average of studied variables between biological fertilizer contents in confidence level of 5%

variances	Df	AC	Pr%
r	2	2.4**	0.29ns
M	3	0.07ns	*0.1
S	3	0.01ns	**1.04
M×S	9	0.2*	**0.62
Error	30	0.08	0.19

*, ** = significant at the 0.05 and 0.01 probability levels, respectively, ns = not significant

Amylose content (AC)

Considering the result of variance analysis and the comparison of the amylose content shows no significant differences between chemical and biological fertilizers (Table7).

Table 8: Results of comparison of average of studied variables between biological fertilizer contents in confidence level of 5%

Variable	AC	PRO%
Year		
2008	22B	9.2A
2009	23A	9.1A
Biological fertilizer		
M1	22.89A	8.99B
M2	22.81A	9.35A
M3	22.92A	9.39A
M4	22.81A	9.19AB
Chemical fertilizer		
S1	22.84A	8.89C
S2	22.89A	9.32AB
S3	22.83A	9.51A
S4	22.87A	9.12BC

M1: no fertilizer, M2: 10 ton cow dung /Ha , M3: 20 ton cow dung /Ha , M4: 5ton azolla compost /Ha
S1: no fertilizer , S2: 40Nkg/ha , S3:60Nkg/ha , S4:80Nkg

with comparison averages review was determined by increasing of N rate in cow dung (M2 to M3) AC reach to the highest content (22.92) whereas in chemical fertilization a slight increase in AC from S1 to

S2, was showed that it will be the highest (22.89) , Also Interaction between chemical and biological fertilizers on the amount of amylose was obtained , AC was the most and least in M1S1 and M4S3 (23/2 and 22/55%) (figure 5). With study results that can be find certainly there is a negative and significant correlation between amount of nitrogen and AC also Ju-Young Lee [3] showed that there is a negative correlation between nitrogen rate and AC. Dong et al. (2007) confirms with the above results. In his research showed that with an increase in the amount of nitrogen fertilizer, activation of starch branching enzymes increased and as a result Amylopectin percentage increases and in contrary AC decreases. Thus a significant negative correlation was observed between the activity of these enzymes and AC. Each time the amount of nitrogen fertilizer increases, enzyme activity will decrease and as a result AC will increase.

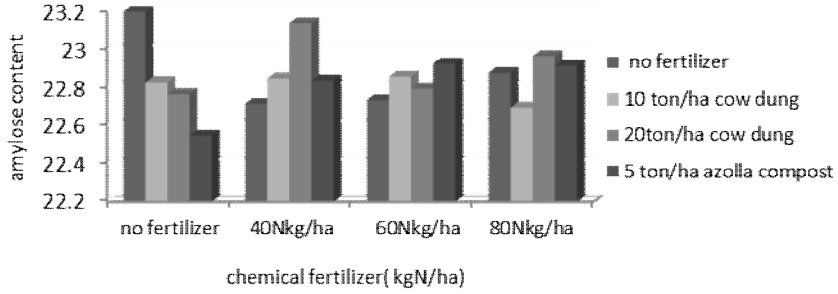

Figure 5: comparison of average of amylase content in contraction between chemical and biological fertilizer

Protein content

Proteins and starch are the two major components of rice, with approximately 8 and 80%, respectively [20,21]. Rice protein is valuable because it has unique hypoallergenic properties and ranks high in nutritive quality (rich in the essential amino acid lysine) among the cereal proteins.Formation of protein in rice grains is closely related to plant nitrogen status and affected by fertilization. [13].

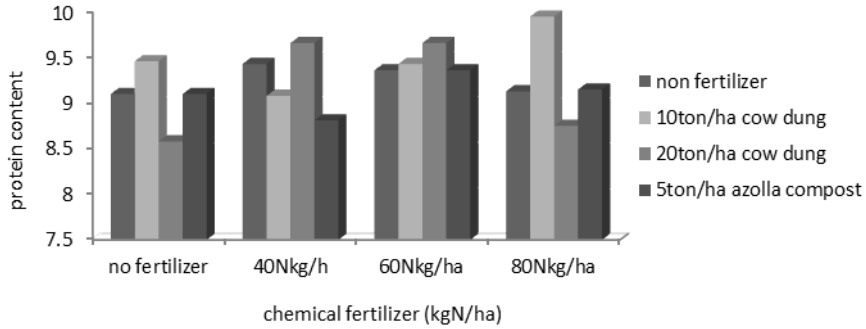

Figure 6: comparison of average of protein content in contraction between chemical and biological fertilizer

In review of another variable, the protein change in the results of analysis of variance at different levels of biological and chemical fertilizers and also assembly of these fertilization show significant differences. this significant difference also appeared in the end results of comparison of average in 5 % confidence level, whereas by increasing of N rate in cow dung from M2 to M3 protein increased as M3 created the highest protein content (9.39%) .Yang et al., [13] showed Formation of protein in rice grains is closely related to plant nitrogen status and affected by variety traits . Although by using of chemicals as a fertilizer protein rate reached to the higher percentage so S3 create the most protein rate. Also HAO et.al [2] showed , The protein content in rice increased with increasing N fertilizer, while amylose content in rice decreased .The research of Chanseoks et .al. in year 2005 showed same as these results there is a positive correlation between protein content and nitrogen fertilizer amount. Also study of interactions shows that the most and the least protein percentage is obtained in M2S4 and M3S1 (9.95 and 8.59%) respectively(figure6) . Also Liu et al. 2005 in his research showed a significant positive correlation between activities of protein synthesizing enzymes and absorption of nitrogen in grain.

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