PYRACLOSTROBIN EFFECT FOR NITROGEN FERTILIZER EFFICIENCY ON CORN (*Zea mays*)

REPORT RESEARCH

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LETTER OF LEGALIZATION

1.	Research Title	:	Pyraclostrobin Effect for Nitrogen Fertilizer Efficiency on Corn (*Zea mays*)
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I. INTRODUCTION

1.1 Corn as C4 Plant

Corn (*Zea mays*) or Maize is categorized as the C4 plant. During photosynthesis, plants use energy from the sun to form sugar from carbon dioxide and water. As temperatures rise, however, photosynthesis becomes increasingly inefficient in most plants and drastically diminishes their productivity during hot weather. However, Corn as the C4 plants have developed an alternate means of photosynthesis that allows them to maintain efficiency, even during hot weather. According several researchers, despite the low numbers of C4 species, they carry out a quarter of the photosynthesis on arable land or agriculture land.

Although most C4 plants grow in the tropics where constant heat gives them an adaptive edge, the Western world's most familiar crop also uses C4 photosynthesis. Indeed, C4 photosynthesis partly accounts for corn's success as a crop. According to the Grain Farmers in America, corn yields double those of most other grain crops, and corn plants also produce twice as much new growth per year as the average temperate forest. As other crops falter under the long days and high heat of midsummer that drives down their ability to synthesize energy for themselves, C4 photosynthesis allows corn to continue efficient energy production, allowing plants to grow and thrive even in the most challenging conditions.

1.2 Corn response to fertilizer

Applying a small amount of fertilizer near the corn seed while planting is a common practice. There are questions, however, about the need for starter fertilizers when soils have high nutrient concentrations, particularly regarding the application of phosphorus (P) in the starter. A number of studies have examined the role of starter fertilizers in corn. Jokela (1992) found a high probability of obtaining a yield response to starter fertilizers containing P and potassium (K) when soil test levels were medium or lower, but the probability of a response was much lower on soils testing higher in P and K. A Missouri study (Scharf, 1999) found an average yield increase of 13 bu/A for plots receiving starter fertilizer when compared to plots with no starter fertilizer in no-till corn; but, he reported that the most profitable starter fertilizer contained only nitrogen (N).

A common assumption among some corn growers is that the application of starter fertilizers containing N will result in more rapid early growth and faster maturity of corn. The application of starter fertilizer did result in visual early growth responses compared to the control plots at all three locations in 2001. These early growth responses were most noticeable on plots containing N in the fertilizer. By the time the corn reached the silking stage, there were significant differences among treatments in the number of days required to reach a given silking percentage. however, there was no consistent trend among the treatments when analyzed as individual sites or when all three sites were pooled together. This lack of consistent trends suggests that the responses were random and that there were no clear differences among any treatments. Hence, further research is still necessary to understand the response.

1.3 Corn Response to Nitrogen Fertilizer

Recent research on corn has tended to show variability in N response. Brown *et al*. (1993) reported that economically optimal N rates among 77 sites in Illinois ranged from zero to more than 200 lb N per acre. Results from other studies show similar variability in time and space. Even with such variability, results over environments have been combined and used to develop an N fertilizer rate guideline in Illinois based on anticipated corn yield (Hoeft and Peck, 2002). This guideline suggests providing 1.2 lb of N (or a different factor based on the relative prices of corn and N) for each bushel of expected yield for corn following corn, with credits given when corn follows a legume or when manure has been applied to the field.

However, we know that yields, and thus actual N requirement, cannot be predicted with accuracy, the use of this guideline has proven to be satisfactory in most years and on most fields. As N costs have increased in recent years, hence, it has become clear that using proven yield as a predictor of N rate tends to result in using more N than can be economically justified. This has led to the use of recent data to formulate N rate guidelines, with adjustments based on N and corn prices (Nafziger *et al*., 2004).

1.4 Pyraclostrobin application

Fungal infections decrease the area of photosynthetic tissue which reduces the transfer of assimilates from their source to the ear and diverts assimilates to fungal growth, defense systems, and increased respiration. Growth stimulation with the strobilurin fungicides has been related to a reduction in the incidence of disease as well as increased nitrate uptake and assimilation in small grains (Kohle *et al*., unpublished). Research has shown that pyraclostrobin was important in stimulating nitric oxide, a key messenger in plants (Conrath *et al*., 2004). Increased nitrate uptake and assimilation following an application of a strobilurin fungicide would justify additional fertilizer at the time of application. Identifying fertilizer sources that synergistically increase yield with a fungicide treatment would provide opportunities to manage disease, reduce application costs, and provide additional fertilizer when crop demand is greatest.

Furthermore, Conrath *et al*., 2004 stated that in several researches shown that *pyraclostrobin* was important in stimulating nitric oxide, a key messenger in plants, increased nitrate uptake and assimilation. Application of a strobilurin fungicide such as *pyraclostrobin* would justify additional fertilizer at the time of application. Identifying fertilizer sources that synergistically increase yield with a fungicide treatment would provide opportunities to manage disease, reduce application costs, and provide additional fertilizer when crop demand is in maximum condition.

II. RESEARCH OBJECTIVE

The objective of this research is : to evaluate the effect of pyraclostrobin application to the nitrogen fertilizer efficiency on corn

III. MATERIAL AND METHOD

This research was conducted at University of Brawijaya Research Station on Jatikerto Village, District of Kromengan, Malang Regency in dry season, from June to November 2011. Research Station had altitude of 235 m above sea level. This research was hold at green house and at field, but the same method applied on them. Although it was consisted of two activities, but all of data arranged and were analyzed at same report.

3.1 Experimental design

This research was conducted using Randomized Complete Block design and three replications. The detail of the design explained on the description follow as:

First Treatment was Pyraclostrobin application (P), consisted of :

P0 : No pyraclostrobin application

P1 : Pryraclostrobin application 400 ml/ha

Second Treatment was Nitrogen application (N), consisted of :

N0 : without N fertilizer

N1 : 30 kg N/ha

N2 : 60 kg N/ha

N3 : 90 kg N/ha

N4 : 120 kh N/ha

3.2 Detailed of Research

Maize cultivation is using the standard manual of Maize cultivation started with Soil tillage, seedling cultivation, fertilizing, pest and weed control. Pyraclostrobin application by foliar spray conducted once on 30 days after planting, with 400 ml/Ha concentration. While Nitrogen Fertilizer was given on 30 days and 40 days after planting each with half of the treatment's dosage. The data was focused on the plant height, number of leave, kernel period, kernel weight, seed weight, protein and amylose content of seed, respectively. While after harvest, amylose and protein analysis was conducted at Food Chemistry Laboratory and Central, University of Brawijaya.

IV. RESULT AND DISCUSSION

Some variables had evaluated, namely plant height, leave number, flowering age, earing age and also amylase and protein content. These data were analyzed using analysis of variance (ANOVA) with randomized block design. The difference among treatments was analyzed by least significant deference. Application of pyraclostrobin 400 ml/ha significantly increase grain amylose until 18.5% higher. Further explanation can be seen on the tables and figures below.

4.1 Vegetative Variables

Generally, it was not vividly significant effect on pyraclostrobin application on vegetative growth of Maize. Surprisingly there is an independent effect between Pyraclostrobin and Nitrogen Fertilizer application. This phenomenon can be seen on Table 1 and 2 for plant height.

Table 1. Plant height for Pyraclostrobin and Nitrogen application on green house

TREATMENT	Plant Age (weeks)		
	4	5	6
Pyraclostrobin (mm/ha)			
0	75.77	99.87	119.10
400	78.69	100.63	118.33
LSD (5%)	Ns	Ns	Ns
Nitrogen (N/ha)			
0	77.39	98.89	117.08
30	76.52	101.5	122.13
60	77.19	97.25	117.75
90	77.54	101.63	120.04
120	77.49	102	116.58
LSD (5%)	Ns	Ns	Ns

Table 2. Plant height for Pyraclostrobin and Nitrogen application on field

TREATMENT	Plant Age (weeks)		
	4	5	6
Pyraclostrobin (mm/ha)			
0	66.55	110.67	116.01
400	66.88	115.11	120.07
LSD (5%)	Ns	Ns	Ns
Nitrogen (N/ha)			
0	63.57	99.06	104.28
30	69.37	114.99	120.33
60	67.43	115.63	120.42
90	67.85	118.71	123.83
120	65.34	116.07	121.32
LSD (5%)	Ns	Ns	Ns

However on the leave number, there was a significant effect of the Nitrogen level increase with the increase of leave number. Nitrogen fertilizer significantly increased the average of leave number, however Nitrogen application did not show significant different of vegetative growth with the increased of its level, as seen on Figure 1 also Table 3 and Table 4.

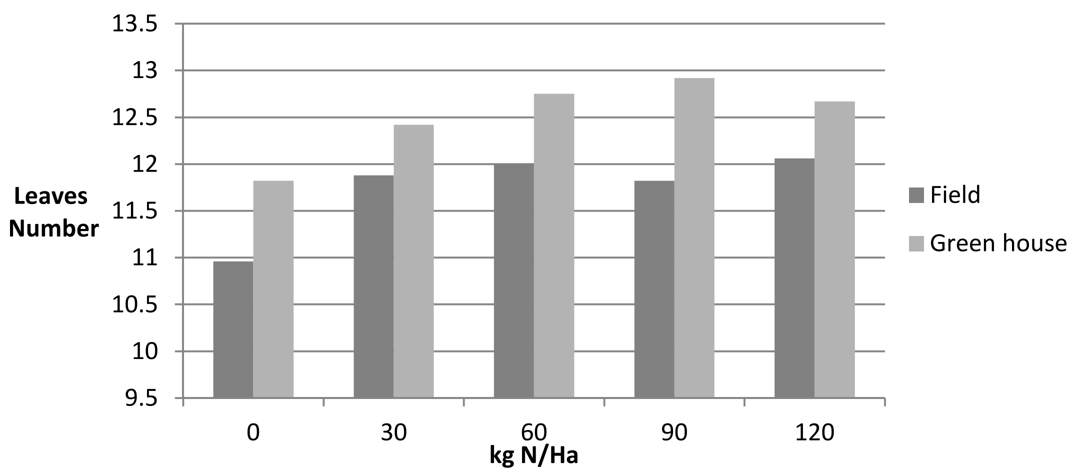

Figure 1. Response of Corn Leave to Nitrogen Fertilizer

Table 3. Leave number for Pyraclostrobin and Nitrogen application on green house

TREATMENT	Plant Age (weeks)		
	4	5	6
Pyraclostrobin (mm/ha)			
0	8.60	11.38	12.54
400	8.53	11.35	12.48
LSD (5%)	Ns	Ns	Ns
Nitrogen (N/ha)			
0	8.33	11.36	11.82 a
30	8.63	11.42	12.42 ab
60	8.63	11.13	12.75 ab
90	8.54	11.38	12.92 b
120	8.71	11.54	12.67 ab
LSD (5%)	Ns	Ns	0,92

Theoretically, early and middle growth of corn, of course, will be affected by nitrogen. Nitrogen was needed for increasing and developing of cells number. But, in this research, application dosage of nitrogen wasn't affect plant length and leave number growth. It didn't known effect of this on other vegetative or physiology characters, like leave length, leave wide, stem diameter, stomata number, etc.

Table 4. Leave number for Pyraclostrobin and Nitrogen application on field

TREATMENT	Plant Age (weeks)		
	4	5	6
Pyraclostrobin (mm/ha)			
0	7.89 a	11.08	11.69
400	8.48 b	11.13	11.79
LSD (5%)	0.60	Ns	Ns
Nitrogen (N/ha)			
0	7.81	10.63	10.96 a
30	8.32	11.42	11.88 b
60	8.22	11.24	12.00 b
90	8.36	11.11	11.82 b
120	8.22	11.15	12.06 b
LSD (5%)	Ns	Ns	0,83

On the other hand, efficiency of nitrogen fertilizer to plant length and leave number wasn't depended on pyraclostrobin application. The corn grew and absorbed nitrogen weren't different from without application pyraclostrobin.

4.2 Generative Variables

Similar phenomenon as vegetative response, showed on generative variables. Both flowering and earing age showed same response with all the treatments. There was no significant earlier or delayed flowering as a response of treatments (Table 5 and Table 6).

Table 5. Flowering and earing age for Pyraclostrobin and Nitrogen application on green house

TREATMENT	Plant Age (weeks)		
	Female	Male	Ear
Pyraclostrobin (mm/ha)			
0	63.49	59.31	62.56
400	62.48	59.10	61.78
LSD (5%)	Ns	Ns	Ns
Nitrogen (N/ha)			
0	63.85	59.07	62.94
30	63.71	59.00	62.92
60	63.08	59.58	62.29
90	62.83	59.17	62.04
120	61.46	59.21	60.67
LSD (5%)	Ns	Ns	Ns

Flowering and earing age of corn didn't determined by pyraclostrobin and nitrogen application. Absorbing nitrogen didn't more efficient if pyraclostrobin applied it, as seen on above tables. Actually, it was not new thing, because nitrogen just absorbed by corn to growing vegetative organ. The absence difference of corn vegetative growth, plant length and leave number, among levels on nitrogen application, made flowering age wasn't different. Of course, it made the earing age wasn't different, too.

Table 6. Flowering and earing age for Pyraclostrobin and Nitrogen application on field

TREATMENT	Plant Age (weeks)		
	Female	Male	Ear
Pyraclostrobin (mm/ha)			
0	60.04	58.15	57.18
400	59.25	57.63	55.77
LSD (5%)	Ns	Ns	2.83
Nitrogen (N/ha)			
0	60.78	58.67	58.06
30	59.96	58.28	56.96
60	59.61	57.57	55.89
90	58.72	57.50	56.10
120	59.17	57.44	55.38
LSD (5%)	Ns	Ns	Ns

Table 7. Ear and seed weight for pyraclostrobin and Nitrogen application on green house and field

TREATMENT	Green house		Field	
	Ear weight (g)	Seed weight (g)	Ear weight (g)	Seed weight (g)
Pyraclostrobin (mm/ha)				
0	118.05	96.32	184.97	150.13
400	125.13	103.43	182.57	152.48
LSD (5%)	ns	ns	Ns	ns
Nitrogen (N/ha)				
0	93.33 a	73.76 a	158.12 a	131.20 a
30	116.63 ab	96.50 ab	187.67 b	156.25 b
60	128.38 b	105.50 b	195.07 b	154.10 ab
90	131.92 b	109.92 b	183.07 ab	151.68 ab
120	137.71 b	113.71 b	194.93 b	163.28 b
LSD (5%)	33.001	30.34	27.84	24.37

But, it was different on yield characters. Nitrogen application showed its effect on weight of seed and ear, which can be seen on Table 7. There was information from this table that nitrogen was absorbed to produce ear and its seed.

Of course, there was different weight of ear and seed on different dosage of nitrogen application. The plant fertilized more dosage of nitrogen capable produce more weight of ear and seed. Figures 2, describe Response of ear and seed weight of corn on nitrogen fertilizer.

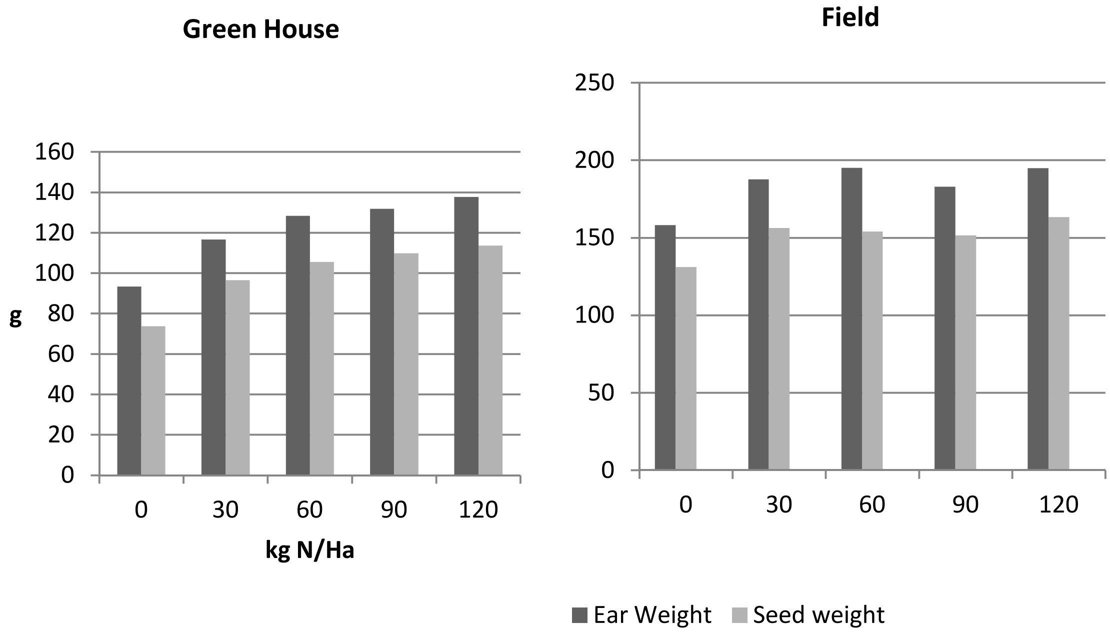

Figure 2. Response of ear and seed weight of corn on Nitrogen Fertilizer

4.3 Amylose and Protein Variables

Regardless of small significance on vegetative and generative variables, it was found that amylose contents was significantly increase with the increase of pyraclostrobin application and Nitrogen fertilizer together or there is a significance interaction between nitrogen and pyraclostrobin to the seed's amylose. The highest application of nitrogen fertilizer together with pyraclostrobin showed the higest amylose content in seed (Figure 3 and Table 8).

Table 8. Amylosa content caused pyraclostrobin applied on green house

Treatment	Amylosa Content (%)
P0N0	18.97 b
P0N1	20.65 cd
P0N2	15.20 a
P0N3	15.99 a
P0N4	15.88 a
P1N0	20.32 c
P1N1	20.81 cd
P1N2	20.71 cd
P1N3	19.43 b
P1N4	21.40 d
HSD (5%)	0,88

There was new information about effect pyraclostrobin on amylose conten of corn. At green house, pyraclostrobin application 400 ml/ha was capable to increase grain amylose content from 17.33% until 20.53% or increase to 18.5% higher. Besides that, pyraclostrobin had the important role to increase the efficiency of nitrogen application. As we seen on Table 8, amylose content increase on the pyraclostrobin 400 ml/ha. Figure 3 support this statement of it.

As we seen on Figure 3, all the treatment with pyraclostrobin shows significant effect on the synthesis of amylose, even with the minimum Nitrogen condition. It is clear that on the small dosage has a positive impact on plant quality (Jabs *et al*., 2000) mainly under stress condition. Further, by foliar application, pyraclostrobin increase plant anti-oxidant level and reduce reactive oxygen in leave tisues by more than 50%. The significant increase of amylose could be also the mechanism of stress tolerance developed by plant with the help of pyraclostrobin.

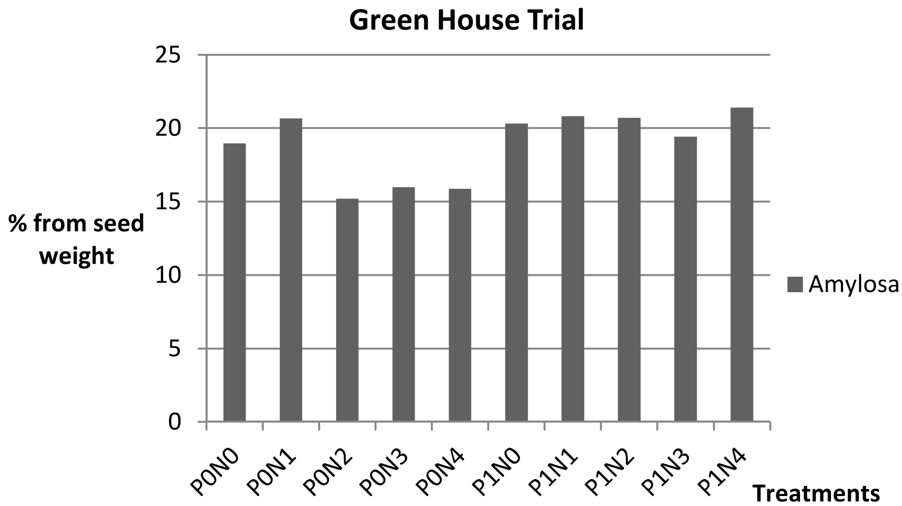

Figure 3. Response of Seed's Amylose content to Pyraclostrobin and Nitrogen

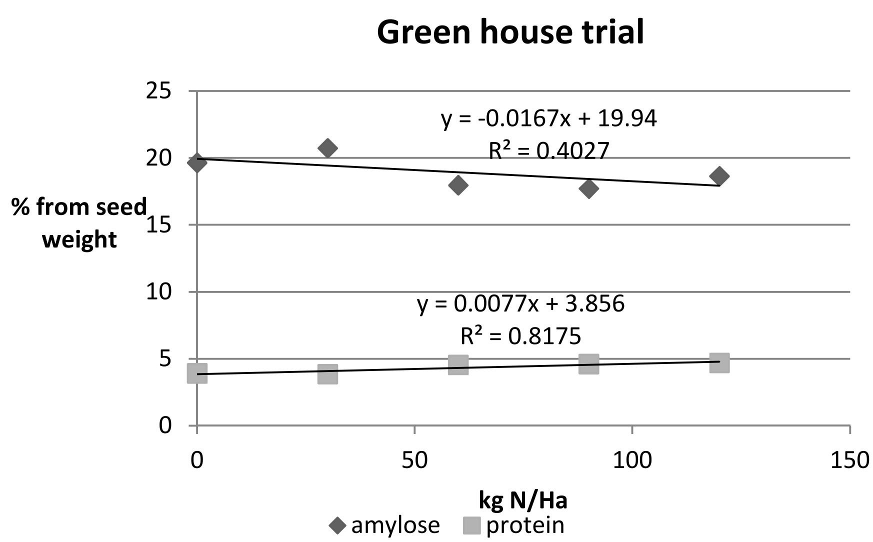

Figure 4. Response of Amylose and Protein Content of Seed to Nitrogen Fertilizer

Figure 4, describes relation formula between nitrogen application with both amylose and protein content at green house. Effect nitrogen to amylosa can be described as straight line y = -0.0167 + 19.94 (0.4027). Effect nitrogen to protein content can be describe as straight line y = 0.0077 + 3.856 (R = 0.8175). Its mean that nitrogen dosage up to120 kg/ha, amylose content will decrease and protein content will increase. But, that is different from field trial (Figure 5). At the field, both of amylose and protein content still increase on 120 kg/ha of nitrogen application.

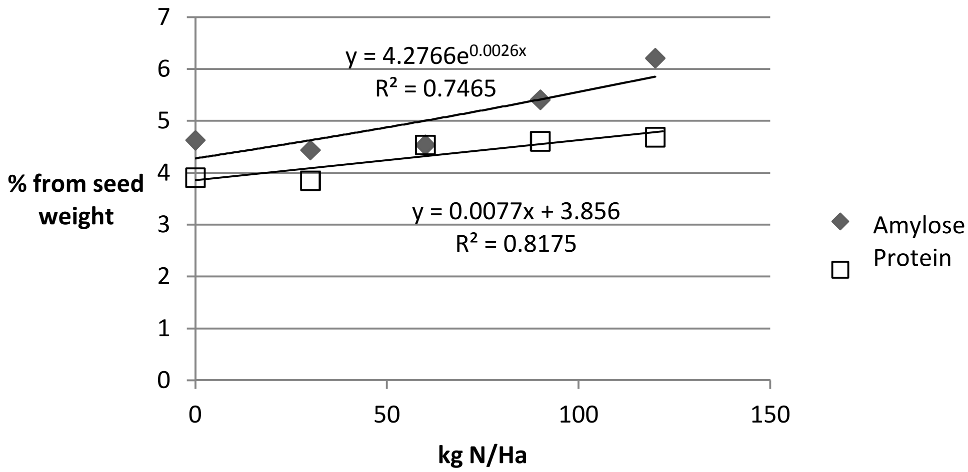

Figure 5. Response of Protein Content of Seed to Nitrogen Fertilizer

On the other hand, on the protein content, only nitrogen fertilizer gave the significance impact on the grain's protein, both at field and green house trial. Table 9, indicate data both of them.

4.4 Nitrogen effect on seed quality

Generally, it is not too dramatizing statement that the application of Pyraclostrobin is change the metabolism in plant. Corn as the C4 plant which is very responsive to Nitrogen fertilizer has showed the alternative response compare to usual response. The increase level of Nitrogen will usually effect on the increase of Protein content in the seed (Morshed *et al.*, 2008). Moreover Morshed *et al.*, 2008 stated that; Nitrogen (N) is often the most limiting factor in crop production. Hence, application of fertilizer nitrogen results in higher biomass yields and protein yield and concentration in plant tissue is commonly increased.

Table 9. Amylose and protein content caused pyraclostrobin applied on green house and field

TREATMENT	Green house		Field	
	Amylose (%)	Protein (%)	Amylose (%)	Protein (%)
Pyraclostrobin (mm/ha)				
0	17.33 a	4.38	19.36	5.01
400	20.53 b	4.25	19.17	5.08
LSD (5%)	0.52	Ns	Ns	Ns
Nitrogen (N/ha)				
0	19.64 c	3.91 a	19.61	4.63 a
30	20.73 d	3.85 a	19.43	4.44 a
60	17.96 a	4.54 b	19.17	4.54 a
90	17.71 a	4.61 b	18.98	5.41 ab
120	18.64 b	4.69 b	19.13	6.21 b
LSD (5%)	0.52	0,57	Ns	1.48

Further, Nitrogen often affects amino acid composition of protein and in turn its nutritional quality. In cereals, abundant supply of nitrogen decreases the relative proportion of lysine and threonine, thus, reducing the biological value of the protein. Increasing nitrogen supply generally improves kernel integrity and strength, resulting in better milling properties of the grain. In oil seed crops, protein levels are increased upon nitrogen fertilization, whereas oil concentration is decreased.

However, effects of nitrogen fertilization on amylose or seed quality are inconsistent. In corn and sugar-beet production, abundant supply of nitrogen results in a reduction of sucrose concentration per unit fresh matter and to an increase in impurities (alpha-amino-nitrogen, invert sugars, and lime salts)

Scharf *et al*., 2002 stated that application of nitrogen fertilizer may increase kernel hardness through increased protein content, and this may be or may not be beneficial to the ethanol industry. Because of the interrelationship between protein bodies and starch granules, an increase in protein may reduce starch extraction. This reduction in starch extraction would not be beneficial to ethanol industry. Hence still there was the negative effect of excessive Nitrogen fertilizer on amylose

V CONCLUSION

1. At green house, application of pyraclostrobin, significantly increase nitrogen fertilizer efficiency. Application of pyraclostrobin 400 ml/ha significantly increase grain amylose until 18.5%.
2. At the field, both of amylose and protein content were increased by nitrogen fertilizer application

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APPENDIX.

1. Middle square observed data at green house

a. Plant height at green house

SV	Df	Week 4	Week 5	Week 6
Replication	2	54.49	468.67	12.19
Treatment	9	18.29	14.32	20.68
P	1	63.95	4.34	4.41
N	4	1.05	26.05	32.29
PN	4	24.12	5.09	13.14
Error	18	42.19	68.38	52.81

b. Leave number at green house

SV	Df	Week 4	Week 5	Week 6
Replication	2	0.28	0.08	0.13
Treatment	9	0.09	0.22	0.54
P	1	0.03	0.01	0.03
N	4	0.12	0.14	1.10
PN	4	0.06	0.35	0.10
Error	18	0.33	0.48	0.29

c. Flowering age and earing age at green house

SV	Df	Flowering age	Male	Earing age
Replication	2	4.97	1.75	3.85
Treatment	9	3.97	0.41	3.90
P	1	7.58	0.33	4.54
N	4	5.45	0.31	5.17
PN	4	1.59	0.53	2.46
Error	18	3.41	0.99	2.97

d. Amylose and protein content, seed and ear weight at green house

SV	Df	Amylose content	Protein content	Seed weight	Ear weight
Replication	2	0.0020	0.64	2344.60	2208.67
Treatment	9	11.07**	0.82**	776.89*	804.58
P	1	51.14**	0.19	379.32	212.89
N	4	6.26**	1.49**	1525.64**	1504.40*
PN	4	5.87**	0.30	127.53	252.69
Error	18	0.09	0.11	312.84	411.11

2. Middle square observed data at field

a. Plant height at field

SV	Df	Week 4	Week 5	Week 6
Replication	2	44.79	20.90	20.21
Treatment	9	33.87	274.24	261.57
P	1	0.80	147.41	123.69
N	4	30.90	370.84	366.94
PN	4	45.11	209.34	190.67
Error	18	15.70	138.83	147.87

b.Leave number at field

SV	Df	Week 4	Week 5	Week 6
Replication	2	0.01	1.16	1.50
Treatment	9	0.51*	0.40	0.68*
P	1	2.65**	0.02	0.07
N	4	0.29	0.52	1.20**
PN	4	0.19	0.38	0.31
Error	18	0.13	0.30	0.24

b. Flowering age and earing age at field

SV	Df	Flowering age	Male	Earing age
Replication	2	2.78	0.13	1.17
Treatment	9	3.04	1.36	5.30
P	1	4.73	2.00	15.05*
N	4	3.69	1.81	6.64
PN	4	1.95	0.74	1.52
Error	18	2.13	0.64	2.73

e. Amylose and protein content, seed and ear weight at field

SV	Df	Amylose content	Protein content	Seed weight	Ear weight
Replication	2	3.65	0.77	913.15	392.70
Treatment	9	0.25	2.02	782.32*	501.96*
P	1	0.27	0.04	42.96	41.54
N	4	0.39	3.44	1389.01**	870.15*
PN	4	0.11	1.10	360.47	248.87
Error	18	0.41	0.75	263.41	201.85

3. Soil analysis (Dry oven 105°C)

a. Before experiment

No. Lab.	Code	pH 1:1		Total N	P.Bray1	K
		H_2O	KCl 1N			NH4OAC1N pH:7
				%	mg kg-1	me/100g
TNH 637	Soil	6.5	5.6	0.07	22.29	0.34

b. After experiment

No .Lab	Code	pH 1:1		Total N	P.Bray1	K
		H_2O	KCl 1N			NH4OAC1N pH:7
				%	mg kg-1	me/100g
TNH 1185	P 0 N 0	6.5	5.7	0.10	15.30	0.10
TNH 1186	P 0 N 1	6.1	5.4	0.10	23.42	0.11
TNH 1187	P 0 N 2	6.2	5.4	0.09	16.98	0.14
TNH 1188	P 0 N 3	6.2	5.4	0.10	20.30	0.45
TNH 1189	P 0 N 4	5.9	5.2	0.09	23.19	0.13
TNH 1190	P 1 N 0	6.3	5.3	0.09	13.87	0.33
TNH 1191	P 1 N 1	6.3	5.4	0.09	23.35	0.50
TNH 1192	P 1 N 2	6.2	5.3	0.09	23.47	0.38
TNH 1193	P 1 N 3	6.1	5.2	0.09	17.11	0.44
TNH 1194	P 1 N 4	6.2	5.4	0.09	18.71	0.43

4. Pictures while research held


Performance of plant at field on 3 weeks (left), and 7 weeks (right)


Performance of plant at green house (left) and field on 7 weeks (right)