Biological control of rice's brown leaf spot caused by *Curvularia lunata* in rice variety IR66 in Cambodia through different treatment methods.

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Abstract

This paper is the first report of blight disease of rice in Cambodia. *Curvularia lunata* (*C. lunata*) has been found to be the cause of brown leaf spot on rice variation IR66. It has become one of the most commonly encountered blight diseases in Cambodia. The symptoms were observed on leaves, spots and blights were brown in color. All isolates were tested for pathogenicity. Bi-culture antagonistic testing showed that *Chaetomium globosum* inhibited sporulation of *C. lunata* to only 41.03 %, and *Chaetomium cupreum* (*Ch. cupreum*) inhibited sporulation of *C. lunata* to 28.55 %, when compared to the control plate. *Ch. cupreum* significantly reduced incidence of brown leaf spot caused by *C. lunata* in a pot experiment. An application of a spore suspension of *Ch. cupreum* to inoculated rice seedlings infected with *C. lunata* reduced disease incidence by 83.96%. An application of biofungicide (ketonium) reduced disease incidence by 87.92%. An application of chemical fungicide (tebuconazole) reduced disease incidence by 86.75%. In the field trial, the chemical method gave the best results in all plant parameters, followed by good-agriculture-practice (GAP) method, then the organic method. The chemical method gave better results in panicles per plant, panicle length (cm), and panicle weight (g) which significantly differed when
compared to GAP and organic method. The chemical method gave the best results in filled grains per panicle, unfilled grains per panicle, grain weight (kg) per plot, dry hay weight (kg) per plot, bio mass weight (kg) per plot, and harvest index (5%). This was significantly better than GAP or organic method.

**Key words:** brown leaf spot, rice, *Chaetomium* sp.

**INTRODUCTION**

Rice belongs to the family of Gramineae, and genus *Oryza*. *Oryzae* contains 20 different species, but only three are cultivated. These are *oryza sativa* L (Asian Rice), *oryza glaberrima* (African Rice), and *oryza japonica* (Japanese Rice). It is the most economically important food crop in many developing countries, and has also become a major crop in many developed countries, where its consumption has increased considerably, particularly in North America and the European Union (EU), due to food diversification and immigration [1].

Rice consumption is vital to the lives of billions of people around the world. Possibly the oldest domesticated grain (10,000 years), rice is the staple food for 2.5 billion people, and growing rice is the largest single use of land for food production, covering 9% of the earth's arable land. Rice provides 21% of global human per capita energy and 15% of per capital protein. Only 6-7% of the world's rice crop is traded in the world market. Thailand, Vietnam, China and the United States are the world's largest exporters. The United States produces 1.5% of the world's rice crop with Arkansas, California and Louisiana producing 80% of the U.S. rice crop, 85% of the rice that is produced in the world is used for direct human consumption [2]. 57% of rice is grown on irrigated land, 25% on rain fed lowland, 10% on the uplands, 6% in deepwater, and 2% in tidal wetlands [3]. Just as rice can be grown in many different environments, it has many characteristics, making one variety more popular in one
region of the world than another \[^4\]. In Cambodia, rice is planted in rainfed lowland areas without irrigation. The total cultivated area for rice production in 24 provinces in 2009 was 2,719,080 hectares (ha) and in 2010 was 2,795,892 ha, but the harvested area in 2009 was 2,674,603 ha and in 2010 was 2,777,323 ha only. The total yield production of rice in 2009 was 7,175,473 tons (t), and average yield was 2.84 t/ha and the total yield production of rice in 2010 was 8,249,452 tons, and average yield was 2.97 t/ha \[^5,6\].

In the last few years, it has been observed that leaf spot disease affects many varieties of rice in Cambodia. It is caused by *Curvularia lunata*. This pathogen not only infects leaves, but also infects rice seeds \[^7\]. The other rice production problem encountered has been the increased use of chemicals including chemical fertilizers and chemical pesticides, and decrease in soil fertility leading to low yields. It has been known that overuse of pesticides causes insects and pathogens to become resistant to chemical insecticides and fungicides, and the toxic residue pollutes the surrounding environment and poses a risk to human beings.

Biological control of plant pathogens has successfully provided a relatively recent strategy for implementation with other control measures. It could reduce the heavy use of chemical fungicides, improving agro-ecosystem and maintain a natural balance. There are several reports on the potential use of biological control agents against plant pathogens \[^8\]. *Chaetomium* species (spp) is one of the strictly saprophytic antagonists against several plant pathogens \[^9\] such as *Phytophthora palmivora* \[^10\] and *Colletotrichum gloeosporioides* \[^11\] and *Pyricularia oryzae* \[^12,13\]. The alternative method is challenging to find the safety agricultural inputs like bio-fertilizer and bio-pesticides to be used instead of those toxic chemicals for the use in rice production in accordance with good agricultural practice (GAP) and organic agriculture \[^14\].

The objective of this research was to analyze ways to eliminate the brown leaf spots caused by *Curvularia lunata* from field cultivated rice variation IR66 in Cambodia, through
the use of diverse control measures (i.e. chemical, GAP, and organic method). Similar research has been performed by Dr. Kasem Soytong et al. in *Bio-formulation of Chaetomium cochlioides for controlling brown leaf spot of rice*[^15] and Huyl Tann et al. in *Comparison between organic, GAP and chemical methods for cultivation of rice varieties in Cambodia*[^16], by using the same control methods, yet this research looks at a different rice variation (i.e. IR66) and seeks to find if there are similar or better positive results yields.

**MATERIALS AND METHODS**

**Isolation and identification of rice pathogen**

Brown leaf spot of rice var. IR66 was isolated from leaf symptom by tissue transplanting method and by placing 10 seeds onto sterilized moist filter paper NO 4 in the petri dish and incubated at room temperature, then periodically observed the fungal structures or fruiting bodies, mycelia etc. under stereo-microscope. The mycelia or fruiting body were then transferred to water agar (WA) for fungal growing for 1-2 days, then transferred onto potato dextrose agar (PDA) until get pure culture. All isolates were identified by morphologically observation under compound microscope.

**Pathogenicity test**

The most frequently found seed-borne fungi were tested for pathogenicity in rice variety IR66. The pathogen inoculum was prepared as spore suspension of 1 X 10^6 spore/ml. The inoculum was inoculated to 20-day-old rice seedlings planted in pots (12 cm dia) by spraying it to the whole seedling, and then covered with plastic bags to keep moist. The number of infected and uninfected leaves per plant were recorded. The two most susceptible varieties of fungi were selected for further experiment.
Bi-culture antagonistic test against rice pathogens

*Chaetomium cupreum* were tested against *Curvularia lunata* causing brown spot on leaves in bi-culture plates. The test was performed by using Soytong’s method\textsuperscript{[14,15]}. Bi-cultures were made of the fungal antagonists and the virulent isolate of *Curvularia lunata* on potato dextrose agar (PDA) and incubated at the room temperature (28-30ºC). The edge of active colony growth of *Curvularia lunata* and *Chaetomium* spp was cut with 0.5 mm diameter by a sterilized cork borer. One agar plug of each fungus was transferred to the opposite sides on the PDA plates of 9 cm diameter and separate cultures of *Chaetomium* spp and *Curvularia lunata* served as controls, then incubated at room temperature (28-30ºC) for three weeks. Data was collected, such as colony diameter (cm) and sporulation, which was counted on Haemacytometer under a compound microscope. The experiment was done using completely randomized design (CRD) with four replications. Data collections such as colony diameter (cm), spore number of tested pathogen, and computed the analysis of variance (ANOV) were recorded, then we compared treatment means using Duncan’s Multiple Range Test (DMRT) at P = 0.05 and 0.01. The experiment was repeated two times.

Efficacy of *Chaetomium* spp to control brown leaf spot of rice var IR66 caused by *Curvularia lunata* in pot experiment

The experiment was done by using randomized complete block design (RCBD) with four replications. Treatments were performed as follows: inoculated (with *Curvularia lunata*) control (T1), inoculated and applied spore suspension of *Chaetomium cupreum* 1 x 10\(^6\) spore/ml (T2), inoculated and applied biofungicide (Chaetomium) 20 g /20 L of water (T3), and inoculated and applied Chemical fungicide (Tebuconazole) 0.1 ml/ 1 L of water (T4). Rice seeds var IR 66 were soaked in purified water for 24 hours and put on moisten paper until germination, then planted into pot (3 seedlings per pot). After rice seedlings were
inoculated for 15 days we perforated the leaves and sprayed with $1 \times 10^6$ spore/ml. This was
done to three wounded leaves/seedlings. Each treatment was applied as mentioned above
every 15 days until harvest. Plant height (cm), number of tillers, fresh and dried root weight
g, fresh and dried panicles (g), grain weight (g) was recorded. The disease index was
modified from Soytong and Quimio’s method$^{[11]}$ as follows: level 1 = healthy, green leaves,
level 2 = 1-10% blighted leaves, level 2 = 11-20% blighted leaves, level, 3 = 21-30% blighted
leaves, level 4 = 31-40% blighted leaves, level 5 = 41-50% blighted leaves, level 6 = 51-60%
blighted leaves, level 7 = 61-70% blighted leaves, level 8 = 71-80%, level 9 = 81-90%
blighted leaves and level 10 = 91-100% blighted leaves. Disease reduction (%) was
calculated based on the formula: (disease index in inoculated control – disease index in
treatment) × 100/disease index in inoculated control. All data was statistically computed, and
the analysis of variance and treatment means were compared using Duncan Multiple’s Range
Test (DMRT) at P=0.05 and 0.01. The experiment was repeated two times.

Application of Chaetomium spp to control brown leaf spot of rice var IR66 in the field
The experiment was performed using randomized complete block design (RCBD)
with 4 replications and 4 treatments as follows: the non-treated control (T1), organic method
(T2), which applied organic fertilizer 4.5kg/plot, liquid biofertilizer 40 cc/20L, bioinsecticide
(Metarhizium and Beauveria) at the rate of 40 cc/20L of water, Biofungicide-Chaetomium
(Chaetomium) at the rate of 10 g/20L of water every 20 days until harvest, GAP method (T3),
which applied the chemical-organic biofertilizer (12-3-3) 1.5 kg/plot. Disease and insect
control were controlled by alternative spraying with bioinsecticide plus biofungicide and
chemical insecticide (Buprofezin 25% WP 30 g/20L) plus chemical fungicide,
(Tebuconazole) 20 cc/20L) every 20 days until harvest, the chemical method (T4) which
applied urea 46-0-0 :at the rate of 0.75kg/plot in early stage and 15-15-15 before flowering
stage at the rate 0.75 kg/plot and spraying with chemical insecticide (Buprofezin 25% WP 30 g /20L) plus chemical fungicide, (Tebuconazole) 20 cc/20L) every 20 days until harvest. The dimension of the individual plot was 6m x 5m (30 m²), but planted area was set up at 5m x 4m(20m2). Each replication was randomly separated by 0.5 m bund. Twenty-days-old seedlings of rice var. IR66 were transplanted in the spacing of 25 × 25 cm. Fertilizer application was done according to the above-mentioned treatments for individual experimental plots. The weed control method for all treatments were done in the same way, by hand .The water management was necessary for rice plantation and affect the yield during seedling stage. Rice plants need less water, so it is not necessary to flood the field but still maintained water in the field. From the panicle initiation phase to the ripening stage the water level is maintained at 5 -10cm, then drained off from the field 10 days before harvesting. For the insect and disease development were observed daily. The harvesting and threshing of the crop from the plot area was done by hand with the help of a sickle. Harvested plants were left in the field for 4-5 days to sun dry. Threshing was done manually, and grains were obtained by winnowing and had a weight of 14% moisture content. The data collected is as follows: plant height (cm), number of tillers per plant, length and weight of panicles, numbers of filled and unfilled grains, grain and straw yields, and bio mass .

RESULT

Isolation of rice pathogen and pathogenicity tests

*Curvularia lunata* was found to be the most present disease in the rice var IR66, collected from either the field or found as a seed-borne fungus. All isolates were tested for pathogenicity to 20-day-old seedlings by inoculating spore suspension at concentration of 1 x 106 spores/ml. Once sprayed, the rice seedlings showed clear symptoms of brown leaf spot. The re-isolation of the plant showed a pure culture of *C lunata*. The most virulent isolate was
then used for further experiments. This research is the first to publicly report blight disease of rice in Cambodia.

**Bi-culture antagonistic test**

Results from bi-culture antagonistic plates at 28 day incubation showed that *Chaetomium* sp could inhibit *Curvularia lunata* in rice var IR66. *Chaetomium* sp had significantly inhibited sporulation of pathogen by 41% when compared to the control plate. Meanwhile, *Ch. cupreum* inhibited sporulation of *C. lunata* by 28.55% when compared to the control plate (Table 1). *Ch. cupreum* significantly inhibited colony growth in bi-culture plate of 21.78% at 28 days which colony diameter of *C. lunata* in bi-culture plate was 7.04 cm when compared to control plate (9.00 cm). *Ch. cupreum* could inhibit sporulation by 28.55% which sporulation of *C. lunata* in bi-culture plate was 183.44 spores/ml when compared to the control plate (256.72 spores/ml) as seen in Tables 1 and 2.

**Efficacy of Chaetomium sp to control brown leaf spot of rice var IR66 caused by Curvularia lunata in pot experiment**

Result showed that *Chaetomium cupreum* can significantly reduce disease incidence of brown leaf spots caused by *Curvularia lunata*. By spraying the spore suspension of *Ch. cupreum*, biofungicide (Chaetomium) and chemical fungicide (tebuconazole) to inoculated rice seedlings with *C. lunata*, the disease can be reduced by 83.96, 87.92 and 86.75%, respectively (table 3). Results showed that *Ch. cupreum* can significantly increase plant height and number of tillers after application of the spore suspension of *Ch. cupreum*, biofungicide (Chaetomium) and chemical fungicide (tebuconazole) to inoculated rice seedlings with *C. lunata*. Plant height significantly increased in treatments of spore suspension of *Ch. cupreum*, biofungicide (Chaetomium) and chemical fungicide.
(tebuconazole) 24.56, 22.79 and 25.24 %, respectively when compared to the inoculated control. The number of tillers also significantly increased in treatments of spore suspension of *Ch. cupreum*, biofungicide (*Chaetomium*) and chemical fungicide (tebuconazole) 34.21, 40.91 and 34.21 %, respectively (Table 4).

**Application of *Chaetomium* sp to control brown leaf spot of rice var IR66 in the field**

The organic, good agricultural practice (GAP) and chemical methods were tested for rice cultivation var IR 66 in the field at Ourong Village, Kouk Tlouk Leu Commune, Chhikhreng district, Siem reap Province, Cambodia. The chemical and GAP methods gave the best results in plant height at 80 days, which were 83.60 cm and 80.90 and followed by organic method (68.70 cm) which significantly differed when compared to non-treated control (68.70cm) as seen in Table 5. Chemical and GAP methods also gave the best results in number of tillers at 80 days which were 14 and 15 tillers, followed by the organic method (12 tillers) which significantly differed when compared to non-treated control (6 tillers) as seen in Table 6. Chemical continued to give the best results in panicle/plant, panicle length (g), panicle weight(g), which were 14 panicle/plant, 26.09 cm, 4.70 g, which differed slightly when compared to GAP which were 14 panicle/plant, 25.38 cm, 4.24 g. The organic method resulted in 11 panicle/plant, 24.83 cm, 3.36 g (Table 7).

The number of filled grain and unfilled grain / panicle, grain and dry hay weight(kg) per plot (20m2) at 14% MC, Bio mass and Harvest Index were gathered. Chemical method gave the best result in filled grain/panicle, unfilled grain/panicle, grain weight(kg)/plot, dry hay weight(kg)/plot, bio mass weight(kg)/plot and Harvest Index (5%) with 111 filled grain, 15 unfilled grain, 10.55 kg, 25.97 kg, 41.04 kg and 0.24, respectively which differed when compared to GAP which were 106 filled grain, 12 unfilled grain, 9.65 kg, 28.49 kg, 35.62 kg.
and 0.23, respectively. The organic method yielded 104 fill grain, and significantly differed with 7 unfilled grain, 6.34 kg, 16.52 kg, 22.61 kg and 0.27, respectively (Table 8).

DISCUSSION

*Curvularia lunata* is one of the most commonly encountered fungal genera, which may infect up to 80 % of seeds and cause grain discoloration [17]. In their article, A new blight disease of rice caused by *C. lunata* from Uttar Pradesh in India, Simon and Lal reported the symptoms were observed from leaves, spots were brown in color, and the maximum infection was recorded in leaf sheath [7].

*Chaetomium cupreum* had been reported to control rice blast pathogen caused by *P. oryzae* in the Philippines [13]. Moreover, the *Ch. cupreum* isolate used in this study is reported by Kanokmedhakul *et al.* [18] found three new azaphilones named rotiorinols A-C (1-3), two new stereoisomers, rotiorin (4) and epi-isochromophilone II (5), and a known compound, rubrorotiorin (6), were isolated from *Ch. cupreum*. Compounds 1, 3, 4, and 6 exhibited antifungal activity against *Candida albicans* with IC50 values of 10.5, 16.7, 24.3, and 0.6 ug/mL, respectively. It is concluded that the control mechanism implies antibiosis.

*Chaetomium cochliodes* (*Ch. cochliodes*) proved to be a new antagonistic fungus against brown leaf spot in rice var Pittsanulok 2 caused by *Drechslera oryzae* in Thailand. It showed good inhibition of mycelial growth of 38.18% and inhibited inoculum production of 71.55%. *Ch cochliodes* was formulated in different forms for applying to control brown leaf spot of rice. Biological products formulated from *Ch cochliodes* were tested to control brown leaf spot of rice caused by *D. oryzae*. Result showed that biopowder formulation gave significantly highest to control leaf spot and highest plant growth when compared to the non-treat control, followed by applying crude extract of *Ch. cochliodes*, benlate and spore suspension of *Ch cochliodes*. Moreover, bio-powder formulation gave significantly increased in plant growth over 44 % and followed by crude extract of *Ch cochliodes*, spore suspension.
of *Ch cochliodes* and benlate. This is reported for the first time for applying *Chaetomium* sp
to control brown leaf spot of rice caused by *C. lunata* in Cambodia.

The chemicals and GAP applications gave better results than the organic method. This
is a contradiction from previous experiments by Tann *et al.*[^19], in which the organic method
gave better rice straw weight than non-treated control, GAP and chemicals, at 115 days of
harvesting. The organic method had an increase plant height and tiller number per plant of
3.06 and 57.69 %, respectively, at 60 days. GAP method increased in plant height and tiller
number of 11.23 and 69.44 %, respectively, while chemical method increased plant height
and tiller number of 6.73 and 62.71 %, respectively. The grain weight (yield) increased in
GAP, chemical and organic methods of 59.15, 55.38 and 44.23 %, respectively. This may be
due to different location of experimental sites, soil fertility, disease and different tested
variety[^20]. The organic method involves many factors for successfully completing the
cultivation[^21].

**Acknowledgements**

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Association of Agricultural Technology in Southeast Asia (AATSEA) and Mr. Boonme Ruengrat, Strong Crop Inter Co. Ltd., Thailand for their partial support of this research
project.
References


Table 1. Efficacy of *Chaetomium cupreum* to inhibit spore production of *Curvularia lunata* at 28 days

<table>
<thead>
<tr>
<th>Number of spore (10^6 cfu / ml)</th>
<th>C.V. (%)</th>
<th>Inhibition(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Bi - Culture</td>
<td></td>
</tr>
<tr>
<td>256.72 a</td>
<td>183.44 b</td>
<td>18.50</td>
</tr>
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</table>
Table 2. Colony inhibition in bi-culture of *Chaetomium cupreum* against *Curvularia lunata* causing brown leaf spot of rice var IR66 at 28 days

<table>
<thead>
<tr>
<th>Colony diameter (cm)</th>
<th>C.V ( % )</th>
<th>Colony inhibition ( % )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Bi - Culture</td>
<td></td>
</tr>
<tr>
<td>9.00 a</td>
<td>7.04 b</td>
<td>2.03</td>
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Table 3 Disease index, disease incidence and disease reduction of rice var IR 66 at 95 days

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Disease index</th>
<th>Disease incidence ( % )</th>
<th>Disease reduction ( % )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non Inoculated control</td>
<td>7.25 a</td>
<td>54.56 a</td>
<td>---</td>
</tr>
<tr>
<td>Spore suspension of Ch. cupreum</td>
<td>2.25 b</td>
<td>8.75 b</td>
<td>83.96</td>
</tr>
<tr>
<td>Biofungicide (ketomium)</td>
<td>1.75 bc</td>
<td>6.59 b</td>
<td>87.92</td>
</tr>
<tr>
<td>Chemical fungicide (Tebuconazole)</td>
<td>2.00 bc</td>
<td>7.23 b</td>
<td>86.75</td>
</tr>
<tr>
<td>C.V.(%)</td>
<td>25.00</td>
<td>39.20</td>
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### Table 4. Plant height at 35 days in pot experiment

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Plant height (cm)</th>
<th>Increased in plant height (%)</th>
<th>Number of tillers</th>
<th>Increased in number of tillers (%)</th>
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<tr>
<td>Inoculated control</td>
<td>14.16 a</td>
<td>-----</td>
<td>3.25 b</td>
<td>-----</td>
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<tr>
<td>Spore suspension of Ch. cupreum</td>
<td>18.77 a</td>
<td>24.56</td>
<td>4.94 ab</td>
<td>34.21</td>
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<tr>
<td>Biofungicide (Chaetomium)</td>
<td>18.34 ab</td>
<td>22.79</td>
<td>5.50 ab</td>
<td>40.91</td>
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<tr>
<td>Chemical fungicide (Tebuconasole)</td>
<td>18.94 a</td>
<td>25.24</td>
<td>4.94 ab</td>
<td>34.21</td>
</tr>
<tr>
<td>C.V. (%)</td>
<td>15.40 %</td>
<td>24.49 %</td>
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</table>
Table 5. Plant Height (PH) in cm at 50 days, 80 days and harvesting days.

<table>
<thead>
<tr>
<th>Varities</th>
<th>Method (TC)</th>
<th>50 days</th>
<th>80 days</th>
<th>Harvesting days</th>
</tr>
</thead>
<tbody>
<tr>
<td>IR66</td>
<td>Non treated control</td>
<td>36.90 c</td>
<td>53.20 d</td>
<td>68.70 c</td>
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<td></td>
<td>Organic</td>
<td>40.50 bc</td>
<td>62.35 c</td>
<td>74.85 b</td>
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<tr>
<td></td>
<td>GAP</td>
<td>47.65 a</td>
<td>67.20 b</td>
<td>80.90 a</td>
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<td></td>
<td>Chemical</td>
<td>43.00 b</td>
<td>72.55 a</td>
<td>83.60 a</td>
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<tr>
<td>CV (%)</td>
<td></td>
<td>4.19</td>
<td>1.71</td>
<td>2.49</td>
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Table 6. Number tiller/plant at 50 day and 80 days

<table>
<thead>
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<th>Varieties</th>
<th>Method (TC)</th>
<th>50 day</th>
<th>80 days</th>
</tr>
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<tbody>
<tr>
<td>IR66</td>
<td>Non treated control</td>
<td>5 c</td>
<td>6 c</td>
</tr>
<tr>
<td></td>
<td>Organic</td>
<td>10 b</td>
<td>12 b</td>
</tr>
<tr>
<td></td>
<td>GAP</td>
<td>11 b</td>
<td>15 a</td>
</tr>
<tr>
<td></td>
<td>Chemical</td>
<td>17 a</td>
<td>14 a</td>
</tr>
<tr>
<td>CV (%)</td>
<td></td>
<td>10.98</td>
<td>9.58</td>
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Table 7. Number of panicles per plant, panicle length(cm) and panicle weight (g)

<table>
<thead>
<tr>
<th>Varieties</th>
<th>Method (TC)</th>
<th>Number of Panicle/plant</th>
<th>Length of panicle(cm)</th>
<th>Panicle weight(g)/panicle</th>
</tr>
</thead>
<tbody>
<tr>
<td>IR66</td>
<td>Non treated control</td>
<td>5 c</td>
<td>23.25 b</td>
<td>2.56 c</td>
</tr>
<tr>
<td></td>
<td>Organic</td>
<td>11 b</td>
<td>24.83 ab</td>
<td>3.36 bc</td>
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<td></td>
<td>GAP</td>
<td>14 a</td>
<td>25.38 a</td>
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<tr>
<td></td>
<td>Chemical</td>
<td>14 a</td>
<td>26.09 a</td>
<td>4.70 a</td>
</tr>
<tr>
<td>CV (%)</td>
<td></td>
<td>9.81</td>
<td>3.30</td>
<td>10.31</td>
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Table 8. Number of filled grain and unfilled grain / panicle, Grain and dry hay weight (kg) per plot (20m²) at 14% MC, Bio mass and Harvest Index (5%).

<table>
<thead>
<tr>
<th>Methods</th>
<th>Filled grain/panicle</th>
<th>Unfilled grain/panicle</th>
<th>Grain weight (kg)/plot</th>
<th>Dry hay weight (kg)/plot</th>
<th>Bio mass weight (kg)/plot</th>
<th>Harvest Index (5%)</th>
</tr>
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<tbody>
<tr>
<td>Non treated control</td>
<td>79 b</td>
<td>16 a</td>
<td>4.35 c</td>
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<td>13.24 c</td>
<td>0.33 a</td>
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<td>Organic</td>
<td>104 a</td>
<td>7 b</td>
<td>6.34 b</td>
<td>16.52 b</td>
<td>22.61 b</td>
<td>0.27 b</td>
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<tr>
<td>GAP</td>
<td>106 a</td>
<td>12 ab</td>
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<tr>
<td>Chemical</td>
<td>111 a</td>
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<td>25.97 a</td>
<td>41.04 a</td>
<td>0.24 ab</td>
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<tr>
<td>CV (%)</td>
<td>9.57</td>
<td>22.73</td>
<td>11.40</td>
<td>13.40</td>
<td>11.86</td>
<td>7.1</td>
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