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# Algal composition in ecosystem of rice field under the application of herbicides and insecticides

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## Abstract

The ecosystem of rice fields is subjected to fluctuations between dry and wet conditions. Therefore, it contains a unique biodiversity of aquatic organisms. The present study was conducted in the rice field to assess the algal composition and changes in algal population after the application of herbicides and insecticides. The physicochemical parameters were measured *in situ* while algal identification and examination were investigated through microscopy. The results showed that there were insignificant changes in terms of water chemistry except for the temperature. Algal examination revealed the composition of 4 phyla (Euglenophyta, Bacillariophyta, Chlorophyta and Cyanophyta) with 18 genera in the rice field. Despite the heavy application of herbicides and insecticides in the rice field, Euglenophyta significantly bloomed in the entire length of study where *Euglena* and *Trachelomonas* were the most dominant genera. Phylum of Bacillariophyta slightly bloomed during control and after the application of herbicides with the most dominant genus was *Nitszchia*. The application of herbicides and insecticides significantly affected the abundance of Chlorophyta even though the total abundance was below than 100 ind/mL. The Cyanophyta were the rarest algae in the ecosystem with only a single genus found, *Oscillatoria*. Conclusively, although the herbicides and insecticides affected the abundance of algae, but it did not induce a shift in algal community. The ecosystem of rice field supports a sustainable growth of Euglenophyta when compared to other phyla.

**Keywords:** Algae, Herbicides, Insecticides, Physicochemical parameters, Rice fields

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## Introduction

The community of algae is highly diversified, and the composition of species varies among aquatic ecosystems. An ecosystem with high domestic loads (high nitrate) was dominated with flagellated algae, *Phacus, Euglena, Chlorella, Strombonas* and *Peridinium* (Sarker et al., 2020). Many species of euglenoids prefer to live in an organic-rich environment with the presence of ammoniumnitrogen (Round, 1981). Meanwhile, Zhang et al. (2021) demonstrated that the algal composition in a freshwater lake, Lake Taihu, changed as a function of temperature where alga population was frequently bloomed during warm temperature. Study on an acid mine drainage have reported that the species diversity decreased with increasing acidity where dominant groups of algae were acidophilic Bacillariophyta (*Eunotia*, *Pinnularia* and *Navicula*) and Chlorophyta (*Mougetia*) (Gomes et al., 2021). In further, Wi´sniewska et al. (2021) found that factors such as irradiance and salinity determined the abundance of algae around the coastal of Southern Baltic Sea. Likely, the physicochemistry of the water varies among ecosystems and therefore affects the algal diversity and algal composition.

The ecosystem of rice fields are unique man-made freshwater ecosystems which are abundant in the South East Asian countries. Rice cultivation is intensively developed considering that rice is a staple food to the local peoples. The plant is cultivated two times per year with a maturity period between 90-140 days depending on the rice species (Che Omar et al., 2019). During the cultivation process, the ecosystems are highly influenced by activities such as fertilizing, pesticide spraying, open burning, and soil liming. In further, the rice ecosystems are subject to wet and drying condition in between of the rice seasons. Since rice is a semi-aquatic plant, the length of wet condition is approximately in 3 months and the depth of water may reach between 3 – 4 inches. This shallow ecosystem may subject between lentic and lotic condition depending on the life stage of the rice plants. During the preparation of the cultivation area, water is introduced into the dry area to soften up the soil ground. Thereafter, ploughing, harrowing and land levelling are required using machineries to till up and aerate the soil. These processes are conducted twice and every time after the processes, the water was flushed out to remove unnecessary elements due to the decomposition and accumulation of solid organic materials. Similarly, in a few weeks after the seed sowing, the plants are exposed to pest attack forcing the farmers to initiate pesticide sprayings. The growth of weed species are also a nuisance and the application of systemic herbicides is an effective solution for weed management. Thereafter, the water is flushed out to get rid all the pesticide residues and a new batch of water is introduced to improve the water quality for the growth of rice plants. Since rice cultivation is subject to systematic water management and changes in water quality, therefore alteration in algae composition is a common characteristic in every rice field.

In the available literature, the composition of algae in rice fields has been reported widely from various geographical locations. In Kartanaka, India which subject to summer and winter season, the rice fields were composed of 71 genera mainly dominated with the group of Bacillariophyta followed by Chlorophyta and Cyanophyta (Jayagoudar et al., 2020). In contrast, the rice fields at Parsa, Nepal were dominated by Cyanophyta more than Chlorophyta (Bala and Sinha, 2017). Chlorophyta was found dominance in the rice fields at Chitral, Pakistan (Ullah et al., 2019) as well as in Lampung Province, Indonesia (Dermiyati and Niswati, 2014). Meanwhile, study on the rice fields around Central Thailand revealed the seasonal appearance of algal composition where Euglenophyta and Bacillariophyta were dominant during dry season but in wet season Chlorophyta was the most abundant algal species (Cochard et al., 2014). In further, the most dominant species in the rice fields of Central Japan was Euglenophyta which also similar with the finding of Dermiyati and Niswati (2014) when cultivating rice plants under organic enrichment greenhouse scheme. Despite the similarity of the land use for rice cultivation across different countries, the algal composition keeps varying and thus, increase the motivation of this study to explore the alga composition in the rice fields of Malaysia.

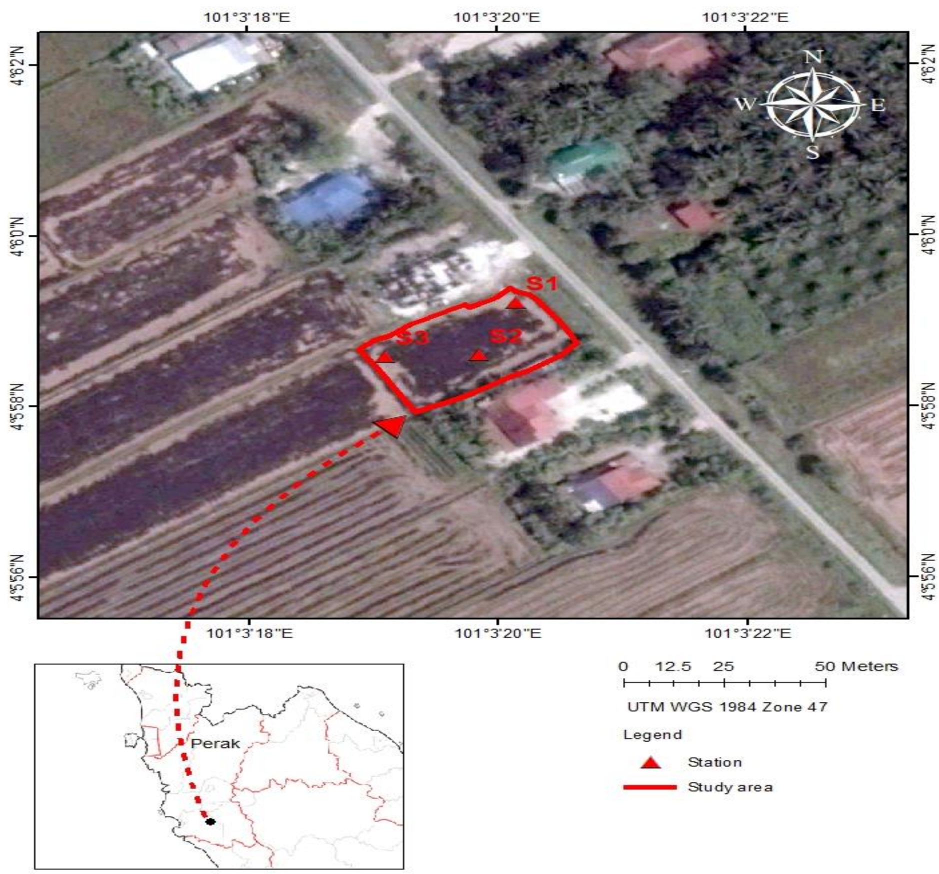
Rice cultivation is among the important agriculture in generating the national economic income in Malaysia. During the cultivation seasons, almost all farmers adopt the pest integrated management to ensure for high yield production. Therefore, the rice ecosystems are heavily exposed with the use of insecticides, molluscicides, fungicides, herbicides, and various types of nutrient-rich fertilizers. It has been proven that the use of pesticides affects the alga communities during laboratory observation (Agirman et al., 2014) or in the fields (De Lorenzo et al., 2001). However, there are considerable differences in the sensitivity of pesticides among the algal species. In analytical review by Roger (1995), the pesticides contain propanil inhibited the growth of several Cyanophyta with concentration of 5 ppm in laboratory observation. However, the same concentration did not produce the inhibition effects in the field investigation. Meanwhile, study by Ma et al. (2006) on 3 species of Cyanophyta and 5 species of

Chlorophyta indicated differential sensitivity of algae on the series of carbamate insecticides: carbaryl, carbofuran, propoxur, metolcarb and carbosulfan. The study also found that the introduction of insecticides resulted with a shift of in algal dominancy. In this study, we aimed to examine the changes in algal composition in the rice field with application of herbicides and insecticides. Some physicochemical parameters were measured as a supporting information on the status of water chemistry. This finding might contribute to the latest information on the algal diversity in rice fields especially during the cultivation season with heavy application of herbicides and insecticides.

## Material and Methods

**Description of the study area**

The study area was situated in a vast rice field at Chenderong Balai, Perak which was located 27 km away from our laboratory at University Technology of MARA Tapah Campus, Perak. The selected study area was located at 04°05’58″ North latitude and 101°03’19″ East longitude within a 38 × 16.5 $m^2$ plain area. The coordinate of area was identified using handheld Global Positioning System (Model: Garmin Rhino) and the map was geo-rectified using the spatial analyst tool, ArcGIS ver 10.0 (Figure 1). Rice is grown by using the direct seeding technique twice per year between September – February and from March – August. This study was conducted in the season between September 2020 – February 2021 which was partly coincided with the Movement Control Order (MCO) due to the pandemic of Covid 19. As a result, some sample analysis and alga identification restrictions were unavoidable.




Station Coordinate:
S1: 4° 5' 59" N 101° 3' 19" E
S2: 4° 5' 64" N 101° 3' 19" E

**Figure-1: Map of the study site**

The sampling began at the end of September (28/09/2020) until the end of November 2020 (22/11/2020). The timing for sampling visits was determined based on the availability of water in the rice field and the timing of operations such as herbicides and insecticides sprayings during the cultivation season (Table 1). The type of herbicides and insecticides with the mode of action were listed in Table 2. The sampling works normally started between 08:00 – 11:00 am i.e., after the sun started to shine and finished before the hot mid-afternoon. There was no interference of rain or flood during every sampling work.

**Table-1: Schedule of activities in the rice field in the duration of study**

| Date | Activities |
|---|---|
| 07 Sept 2020 | First water introduction after a long drying period |
| 13 Sept 2020 | Soil harrowing and ploughing. Thereafter, the water was flushed out. |
| 14 Sept 2020 | Second water introduction |
| 20 Sept 2020 | Second time soil ploughing, soil harrowing and soil levelling. Water was flushed out, thereafter. |
| 21 Sept 2020 | Third water introduction |
| 30 Sept 2020 | Seed sowing activities |
| 6 Oct 2020<br>12 Oct 2020 | Seed germination<br>First sampling work (Control condition)<br>Growth of seedlings |
| 18 Oct 2020 | Herbicides spraying |
| 23 Oct 2020 | Second sampling work<br>Fertilizing process |
| 12 Nov 2020 | Insecticides sprayings |
| 22 Nov 2020 | Third sampling work |
| | Water was flushed out to allow the |

| | |
|---|---|
| | ripening process of the rice plants |
| 15 Jan 2020 | Rice harvesting season |

**Determination of physicochemical parameters and sample collection**

The pH, temperature and total dissolved solid (TDS) were measured in the water system of the rice field as part of a physicochemical study. At each sampling visit, the parameters were measured in situ with a portable multiprobe meter (Model Testr™ 35). The readings were repeated in triplicate. Temperature and TDS were indicated as °C and ppm, respectively.

Since the deepest part of the rice field was less than 0.5 m, samples of water were directly scooped to capture the alga sample using 200 mL plastic bottle. The algae sample was added with a few drops of 4% formalin for preservation where the formalin could eliminate decomposer organisms and cease the zooplankton grazing activities. Samples were quickly transported to Plant Laboratory in University Technology of MARA, Tapah Campus for further investigation.

**Table-2: Description of herbicides and insecticides applied on the rice field during the duration of study.**

| | Type of chemicals | Active ingredient (ai) and dosage of application | Mode of action |
|---|---|---|---|
| Herbicides | Sofit N300EC | Pretilachlor 28.7% w/w Safener Dosage: 1000ml/hectare | Pre-emergence selective herbicides. Absorb readily by the roots of germinating weed. Cell division inhibitor. |
| | Gamit 53.1 | Clomazone 17.7% w/w, Propanil 35.4% w/w Dosage: 40-60mL/10L water | Pre-emergent selective herbicides to control broadleaved, grass, and sedge weed species. Inhibition of photosynthesis |
| Insecticides | Decis 250 | Deltamethrin 2.8% Dosage: 250mL/hectare | Modification of the sodium channel kinetics leading to hyperexcitation of the nervous system Induces neurotoxicity and the effects of deltamethrin on nervous, respiratory, and hematological systems in organisms |
| | Prevathon | Chlorantraniliprole 400ml/hectare | Acts as a selective activator for ryanodine receptor Impedes the insect's ability to regulate muscle function and leads to permanent muscle contraction. |

*** 1kg ai per hectare yielded for 1-2 ppm in water (Roger, 1995)

**Algae identification and algae examination**

Taxonomic algae identification was determined by preparation of wet mount slide observed under optical light microscope (Olympus) attached with camera and image analysis software (Infinity 2 Lumenera). Total magnification for identification was selected under 100×, 200× and 400× depending on algal size. Identification of algae was conducted up to the genus level based on references from Prescott (1951), Salleh (1996) and Shamsudin (1991).

The algae were examined using a sedimentation tube according to the Utermöhl method (Utermöhl, 1958). 1 mL of water was aliquoted into the tube, and the preparation was left for one hour to allow for complete algal sedimentation. The slide was next examined under an inverted microscope (Olympus). Individual algae were counted under the total magnification ranging from 200× to 400× depending on the size of the algae. The algal population abundance was determined by the number of algae, and the unit was given as ind/mL.

**Statistical analysis**

To determine the differences in algal composition and physicochemical properties among agricultural operations (application of herbicides and insecticides), a statistical analysis was undertaken using IBM SPSS Statistics 25. Prior to doing parametric tests such as one-way ANOVA and post hoc testing, the data was examined for normality assumptions. Data on pH were normalised using reciprocal transformation due to a violation of the normality assumption. Natural logarithm ($Lg_{10}$) transformation was used to standardise data on population abundance for Euglenophyta and Chlorophyta. For post hoc comparison, the Tukey HSD test was chosen, and all analyses were set to $P < 0.05$. The data on Cyanophyta were removed for statistical analysis since the total number of individuals in Cyanophyta was fewer than 10 and they did not consistently appear during the sampling visits. To determine algal dominancy, data on population abundance was analysed independently in each agricultural activity. Data on the population abundance of each algal phylum was also analysed independently throughout the agricultural activity to determine the impact of herbicides and insecticides.

## Results

### Physicochemical parameters (Temperature, pH, and TDS)

In overall, the range of water temperature was within 24.00 to 25.50°C in the entire sampling visits from September until December 2020. In details, the water temperatures were significantly different during control and after the application of herbicides and insecticides (one-way ANOVA; $P < 0.05$; Table 3). However, the highest water temperatures were found after the application of herbicides but were not significantly difference with control condition (Tukey test; $P > 0.05$; Figure 2). The lowest water temperatures were significantly displayed after the application of insecticides (Tukey test; $P < 0.05$; Figure 2). Meanwhile, the range of pH and TDS were between 6.00 to 6.80 and 40 to 70 ppm, respectively. Both parameters did not reveal any significant differences in the entire sampling visits (one-way ANOVA; $P > 0.05$; Table 3).

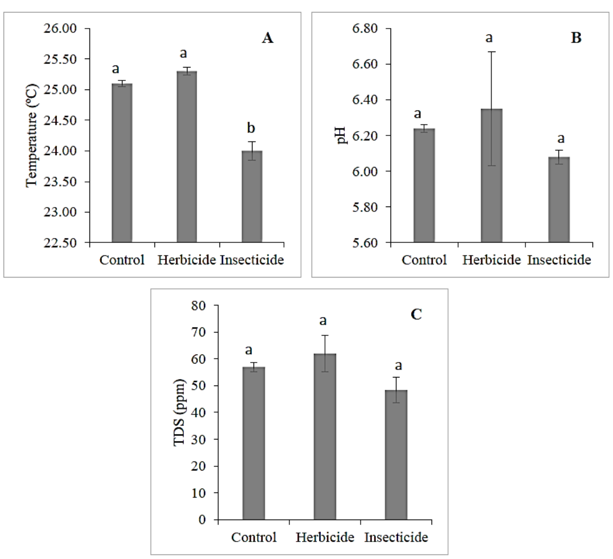



**Figure-2: The readings of A: temperature (°C), B: pH and C: TDS (ppm) during control condition and after the application of herbicides and insecticides. Letter above bar denotes for post hoc Tukey HSD test at $P < 0.05$.**

**Table-3: The one-way ANOVA analysis for physicochemical parameters across the duration of study.**

| | SS | df | MS | F | Sig. |
|---|---|---|---|---|---|
| **Temperature (°C)** | | | | | |
| Between group | 2.842 | 2 | 1.421 | 16.610 | 0.004* |
| Within group | 0.513 | 6 | 0.086 | | |
| Total | 3.356 | 8 | | | |
| **pH** | | | | | |
| Between group | 0.000 | 2 | 0.000 | 0.116 | 0.893 |
| Within group | 0.001 | 6 | 0.000 | | |
| Total | 0.001 | 8 | | | |
| **TDS (ppm)** | 283.868 | 2 | 141.934 | 0.657 | 0.552 |
| Between group | 1296.494 | 6 | 216.082 | | |
| Within group | 1580.363 | 8 | | | |
| Total | | | | | |

**significant value at p<0.05*

**Table-4: Algal composition in the ecosystem of rice field under different agricultural conditions**

| Algae taxa | Control | After herbicide spraying | After insecticide & fertilizing |
|---|---|---|---|
| **Euglenophyta** | | | |
| *Euglena* | ++++ | +++ | +++ |
| *Trachelomonas* | ++ | ++++ | ++++ |
| *Phacus* | ++ | + | ++ |
| **Bacillariophyta** | | | |
| *Nitzschia* | +++ | +++ | ++ |
| *Navicula* | ++ | ++ | - |
| *Cymbella* | + | - | - |
| *Surirrella* | + | - | - |
| *Neidium* | - | ++ | - |
| *Pinnularia* | - | + | + |
| *Synedra* | - | + | - |
| *Tabellaria* | - | + | + |
| **Chlorophyta** | | | |
| *Chlamydomonas* | ++ | - | - |
| *Closterium* | ++ | - | - |
| *Pandorina* | + | + | - |
| *Scenedesmus* | + | ++ | + |
| *Cosmarium* | + | + | + |
| *Staurastrum* | - | - | + |
| **Cyanophyta** | | | |
| *Oscillatoria* | - | + | + |
| Total no of genus | 12 | 13 | 10 |

*Indication notes: ++++ >500 ind/mL, +++ <100-500 ind/mL, ++ <50-100 ind/mL, ++ <10-50 ind/mL, + <10 ind/mL*

### Algal composition and algal dominancy under different agricultural conditions

The ecosystem consisted of 4 main phyla with 18 genera (Table 4). Euglenophyta consisted of 3 main genera dominated by *Trachelomonas*, *Euglena* and

*Phacus* in respective sequence (Figure 3). The Bacillariophyta contained the highest diversity of genera with the genus of *Nitzschia* (Figure 3) was more dominant followed by *Navicula*. Other genera were found in rare number (< 5 ind/mL). In Chlorophyta, the genus of *Chlamydomonas* was dominant but only occurred during control condition and disappeared after herbicides and pesticides application. Other genera of Chlorophyta existed in rare number (<10 ind/mL). The rarest phylum found in all conditions was Cyanophyta which only composed of a single genus, *Oscillatoria* (<10 ind/mL).

In every condition, the population abundance was significantly difference across the algal phyla (One-way ANOVA; $P < 0.05$; Table 5). The Euglenophyta significantly displayed the highest population abundance under control condition and after the application of herbicides and insecticides (Tukey test; $P < 0.05$; Figure 4). However, the population abundance of Euglenophyta was not significantly different with Bacillariophyta after the application of herbicides (Tukey test; $P > 0.05$; Figure 4). The lowest population abundance was significantly found in Bacillariophyta, Chlorophyta and Cyanophyta during control and after the application of insecticides (Tukey test; $P < 0.05$; Figure 4).

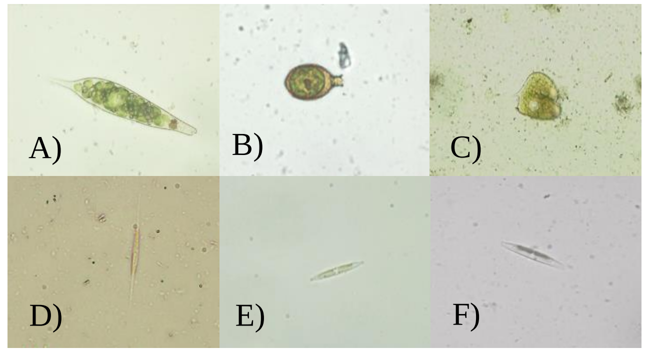



**Figure-3: Micrograph on some dominant algal species found in the rice field. A) *Euglena* sp. B) *Trachelomonas* sp. C) *Phacus* sp.; D-F) variant of *Nitzschia* sp.**

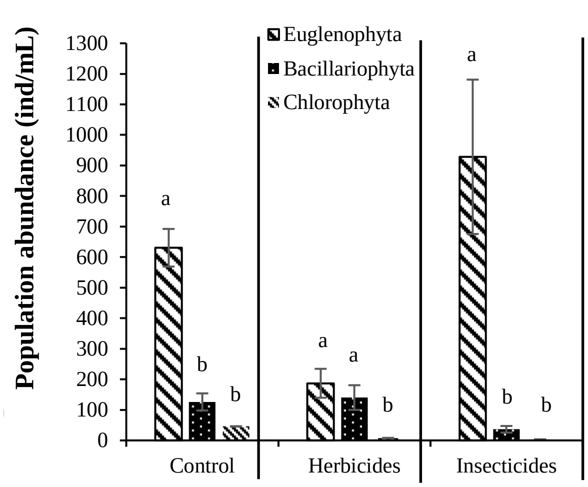



**Figure-4: The algal dominancy based on population abundance (ind/mL) across algal phyla in control condition and after the application of herbicides and insecticides. Letters above bar denote for post hoc Tukey test.**

**Table-5: Statistical analysis using One-way ANOVA on the difference of population abundance across the algal phyla in control condition and after the application of herbicides and insecticides.**

| Activities | SS | df | MS | F | Sig. |
|---|---|---|---|---|---|
| **Control** | | | | | |
| Between group | 1.957 | 2 | 0.978 | 32.210 | 0.001* |
| Within group | 0.182 | 6 | 0.030 | | |
| Total | 2.139 | 8 | | | |
| **Herbicides** | | | | | |
| Between group | 2.864 | 2 | 0.000 | 0.116 | 0.016* |
| Within group | 0.970 | 6 | 0.000 | | |
| Total | 3.834 | 8 | | | |
| **Insecticides** | | | | | |
| Between group | 9.178 | 2 | 4.589 | 23.999 | 0.001* |
| Within group | 1.147 | 6 | .191 | | |
| Total | 10.325 | 8 | | | |

## Impact of herbicides and insecticides on each algal phyla

The application of herbicides and insecticides did not affect the population abundance of Euglenophyta and Bacillariophyta (One-way ANOVA; $P > 0.05$; Table 6) except for Chlorophyta (One-way ANOVA; $P < 0.05$; Table 6). The population abundance of Chlorophyta was the highest during control condition but later, was significantly reduced after the application of herbicides and insecticides (Tukey test; $P < 0.05$; Figure 5).

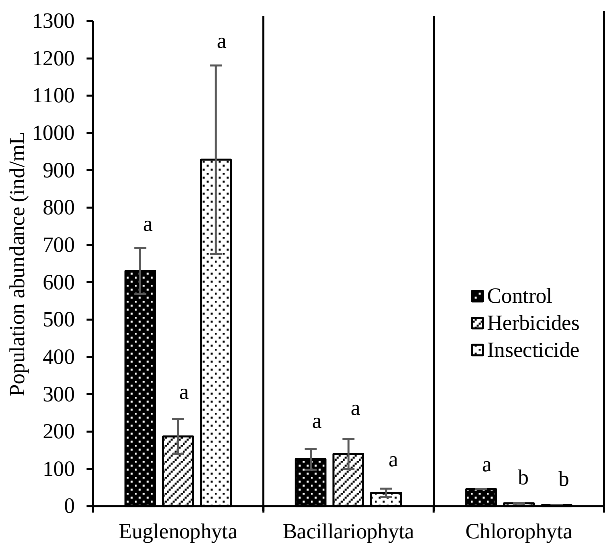
**Figure-5: The population abundance (ind/mL) of algal phyla in comparison to control condition and after the application of herbicides and insecticides. Letters above bar denote for post hoc Tukey HSD test**.

**Table-6: Statistical analysis using One-way ANOVA for each algal phylum to compare the difference between population abundance across control condition and after the application of herbicides and insecticides.**

| Algal phyla | SS | df | MS | F | Sig. |
|---|---|---|---|---|---|
| **Euglenophyta** | | | | | |
| Between group | 0.935 | 2 | 0.468 | 3.626 | 0.093 |
| Within group | 0.774 | 6 | 0.129 | | |
| Total | 1.709 | 8 | | | |
| **Bacillariophyta** | | | | | |
| Between group | 19061.556 | 2 | 9530.778 | 1.253 | 0.351 |
| Within group | 45627.333 | 6 | 7604.556 | | |
| Total | 64688.889 | 8 | | | |
| **Chlorophyta** | | | | | |
| Between group | 2.436 | 2 | 1.218 | 27.297 | 0.001* |
| Within group | 0.268 | 6 | .045 | | |
| Total | 2.704 | 8 | | | |

## Discussion

Rice fields are well known for their changing ecosystems between dry and wet conditions. The ecosystem is exposed to systematic water management during wet conditions, with the water level being managed according to the life stage of the plants. However, the wet condition supports for growth of many organisms. Investigation on the ecosystem of rice field at Chenderong Balai, Perak revealed the trend of physicochemical properties and algal composition during the cultivation season. The impact of herbicides and insecticides were analysed on the algal composition and each of algal phyla.




### Physicochemical characteristics and algal composition in the rice field

Investigation on the physicochemical parameters revealed that the entire study area was a homogenous ecosystem based on the pH and TDS readings. The pH values between 6.00 to 6.80 were considered as suitable for rice cultivation (Azman et al., 2014). Meanwhile, the variation in water temperatures was purely a function of rice plant growth. During the conditions of control and herbicides application, the rice field was characterized with an open water ecosystem where the rice plants were in the early stages of growth (seed germination and rice seedlings). Therefore, allow the light penetration directly on the water surface. However, as the seedlings matured into adult plants, they formed a canopy above the water surface, lowering the water temperature reading.

In overall, a total of 18 genera from 4 different phyla were identified from the rice field by qualitative and quantitative analysis. Euglenophyta appears to be the most dominant group of algae. This population was noticeably bloomed in the beginning of season (control) and later, the highest blooming scenario was observed after the application of insecticides. According to Graneli and Turner (2006), an algal community is considered blooming when the cell abundance reaches hundreds to thousands of cells per millilitre.

In this study, the phylum of Euglenophyta bloomed in the entire season and likely, the water chemistry might be one of the reasons. There were some studies reported that the bloom of euglenoids in respect to physicochemical factors. Study by Rahman et al. (2007) showed that the bloom of euglenoid was coincided with water temperature above 30°C and the pH was around 6.0. However, an extensive study by Ratha et al. (2006) reported the bloom of euglenoids at lower temperature between 26.0-30.0°C and at higher pH range around 7.0-9.0 in various aquatic habitats. In other study, euglenoids did not even bloomed in the range of temperature between 26.0-35.0°C and pH around 6.8-7.3. In further, their study also showed the TDS values were around 51.0-58.0 ppm (Parisara and Narayana, 2016). Our study showed the bloom of euglenoids occurred at much lower temperature ranging from 25.0 to 25.5°C and pH within 6.00 to 6.80, while TDS values were recorded between 40.0 to 70.0 ppm. Apparently, there are inconsistent findings to relate between the physicochemical parameters and the bloom of euglenoid. Therefore, may suggest for the presence of other regulating factors in the water content.

In literature, we found that the bloom of Euglenophyta in the rice field ecosystems of Japan was coincided with the addition of fertilizer and compost (Yamazaki et al., 2001). In both situations, the rice fields were also treated with similar regimes of herbicides and insecticides. In the rice field of Indonesia, the Euglenophyta turned to be dominant with the addition of organic compost 'bokashi' (Dermiyati and Niswati, 2014). However, during the rice cultivation season, the farmers in Indonesia were using natural insecticides made from tobacco and ginger instead of chemical insecticides mainly practiced by the farmers in Malaysia and Japan. We also made a comparison with the findings of Euglenophyta in places other than the rice fields. The Euglenophyta was dominant in the homestead ponds loaded with nutrient from household outputs (Sarker et al., 2020) and in the municipal slow-flowing Zasavica River in Serbia (Predojević et al., 2015). In fact, this group was considered as a biological indicator for ecosystems with organic-polluted water (Patil et al., 2021). Therefore, we predict that our study area may contained a high amount of organic matter and high nutrient concentration where the Euglenophyta is capable to utilize the sources more successful than the other phyla.

**Effects of herbicides application on algal phyla**

Although the phylum of Euglenophyta was significantly unaffected across every condition, we noticed a trend of reduction after the application of herbicides. A significant reduction was significantly demonstrated by the Chlorophyta even though this phylum was not blooming in every condition. The types of herbicides used during the study period contained active ingredients such as pretilachlor, clomazone and propanil (Table 2). The addition of safener was intended to protect the effect of herbicides on the rice plants while leaving the dicot weed species harmed by the herbicides. These were pre-emergence selective herbicides targeted on weed species commonly grown during the cultivation season with the mode of action to prevent the plant's cell division and interfere the photosynthesis pathway (De Lorenzo et al., 2001). Although the use of such herbicides was to combat the growth of weed species, however it has noticeable systemic impacts to non-target photosynthetic organisms such as algae in the rice field.

In the previous studies, pretilachlor showed negative effects on the blue-green algae, *Synechocystis* by decreasing the photosynthesis rate, inhibiting the growth rate and reduced the nitrogen assimilation (Singh et al., 2016). Another similar group of chloroacetamide herbicide, alachlor reduced the densities of many species of Chlorophyta (*Scenedesmus, Ankistrodesmus*, *Crucigenia* and colonial green algae) and Bacillariophyta (*Nitzschia* and *Melosira*) in an agricultural stream. According to Chang et al. (2011) and Ma (2002), herbicides toxicity affected the green algae more than the other algae. In addition, algae with high chlorophyll a content were more vulnerable to photoinhibition than species of euglenoids. The reason that euglenoids can sustain under photoinhibition is by switching the mode of nutrition from autotrophic to heterotrophic feeding (Perschbacher and Ludwig, 2004; Debenest et al., 2009). This evidence was collected from both laboratory observations and field investigations which provide a direct explanation to highlight our findings. After application of herbicides containing multiple active ingredients such as pretilachor, clomazone, and propanil, we found that some genera from Chlorophyta (*Chlamydomonas* and *Closterium*) have disappeared and the bloom of Euglenophyta was affected as well. However, statistical analysis did not reveal any significant differences on the abundance of Euglenophyta. This finding suggests for further laboratory testing to verify the toxicity of herbicides on species-specific in future study.

Another interesting discovery was detected when Bacillariophyta bloomed at equal abundance with Euglenophyta after herbicides spraying. In contrast, the Bacillariophyta remained in lower abundance than Euglenophyta at control and after the application of insecticides. Naturally, euglenoids are motile and the fact that they can switch their mode of nutrition from photosynthetic to heterotrophic is an advantage to exploit the rice ecosystem. Leedale (1967) stated that heterotrophic euglenoids were major consumers to phytoplankton especially the group of Bacillariophyta (diatoms). When the toxic effect of herbicides renders the dominancy of euglenoids, this effect may allow the bloom of diatoms in this study area. The blooms between euglenoids and diatoms are common indication in nutrient-rich ecosystem and population increases and correlation between these groups were explained in detail by Duttagupta et al. (2004) and Rahman et al. (2007).

**Effects of insecticides application on algal phyla**

Toxicity of herbicides causes for reduction in the population of Euglenophyta but thereafter, has successfully revived and bloomed even higher. It seems that the insecticides spraying did not affect further on the population of euglenoids. However, the population of other microalgae were still in the minority. Cochard et al. (2014) found that the application of strong synthetic insecticides in the rice fields of Central Thailand did not affect the euglenoids, but the algae was more affected with the application of natural insecticides containing azadirachtin, saponins, phytoalexins, tannins, and alkaloids (Mulderij, 2006; Petroski and Stanley, 2009; Jančula et al., 2010). In our study, the farmers applied both synthetic and natural insecticides simultaneously with active ingredients chlorantraniliprole (Prevathon) and deltamethrin (Decis 250). These insecticides act on a wide range of pests such as Lepidoptera, Coleoptera, Diptera, Isoptera and Hemiptera (Wang and Wu, 2012; Wan Jaafar et al., 2013). The mode of actions and the dosage used for each pesticide are described in Table 1.

The study on the impact of insecticides to algal groups is widely documented but information on the similar insecticides utilized in this study is relatively scarce. Thus, making the detail comparison is limited. Basically, insecticides act on the nervous system and the muscle system of insects which might leave the group of algae unaffected. However, some studies stated that the toxicity of chlorantraniliprole (Prevathon) in green algae, *Pseudokirchneriella subcapitata* was induced at concentration of more than 4.0 ppm within 72 hours of exposure (United States Department of Agriculture, 2019). Similarly, the active ingredient in Decis 250, deltamethrin, is capable to harm the population of algae too. In the laboratory observation, deltamethrin inhibited the growth of blue-green algae, *Calothrix* from 30-70% with concentration of 17-140 ppm (Gupta and Baruah, 2020). However, deltamethrin with low concentration of $2.0 \times 10^{-5}$ ppm did not produce inhibitory effect on the growth rate of green algae, Chlorella vulgaris within 14 days of exposure (Lutnicka et al., 2014). The review by Roger (1995) stated that application of 1 kg of active ingredient approximately yielded the concentration between 1-2 ppm of pesticides in the water content. In relative, at our study site, the farmers were spraying low dosages insecticides which yielded approximately 0.37 ppm and 0.60 ppm of deltamethrin and chlorantraniliproles respectively. Therefore, the impact of insecticides on the algae was undetectable.

Similarly, Roger (1995) emphasized that the influencing factors for toxicity of pesticides was not only dependent on the applied dosage but also due to the persistency of the pesticides in the environments. Basically, the half-life of chlorantraniliprole was 1.36 days following its application at recommended dosages (Kar et al., 2013). The fastest half-life was demonstrated by deltamethrin with 8-48 hours within aqueous phase (Erstfeld, 1999) and water system contained soil microbes enhanced the degradation process (Ismail et al., 2015). In our case, we conducted the sampling work 10 days after the application of insecticides where the time interval possibly enough for degradation of the compound in the rice field. To understand the detail impact of insecticides, further investigation is required in the laboratory condition on species-specific.

## Conclusion

Based on the most physicochemical indicators used in this study, the rice fields appeared to be a homogeneous environment. The phylum Euglenophyta remained as the most dominant and blooms in the entire season despite the heavy application of herbicides and pesticides. This scenario may suggest further investigation on the nutrient content of the water chemistry. The phylum Bacillariophyta was slightly bloomed but the blooming was not sustainable after the insecticides's application. The herbicides' application affected Chlorophyta, but other phyla remained unaffected significantly. Apparently, the ecosystem did not induce a shift in either algal composition or algal dominancy. These findings are based on the field investigation and require further investigation primarily on the nutrient analysis and quantitative analysis on the organic matter and pesticides in the water content. The role of Euglenophyta in controlling the presence of other algae is an interesting subject in future study considering their ability to switch their mode of feeding under photoinhibition effects due to herbicides application.

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## Contribution of Authors

Ismail HN: Conceived idea and designed the experimental study, ran the field works and laboratory analysis, and prepared the manuscript
Noor NM: Assisted in identification of algae, academic writing and reviewing process
Ahmad Z: Assisted in determination of location and prepared the map of the study site using ArcGIS 10 and academic writing
Wan Anuar WNH: Assisted in data collection during field work and laboratory analysis