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"Runoff of pesticides into the aquatic environment - potential hazards for microalgae?"

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Abstract

Pesticide runoff represents a notable risk to aquatic primary producers, yet the effects of chemical stress in combination with light intensity on microalgae remain poorly understood. We examined three commonly used pesticides—the herbicide Basar, the fungicide Ortiva, and the insecticide Teppeki—on the growth, pigment allocation, and photosynthetic performance of the green microalga *Chlorella vulgaris* and the cyanobacterium *Synechococcus leopoliensis*. Basar contains the active ingredient S-metolachlor, Ortiva contains azoxystrobin, and Teppeki contains flonicamid as the active substance. Cultures were exposed to highly diluted pesticide solutions for seven days under weak and strong irradiance. Physiological responses were measured using optical density (OD), in vivo chlorophyll a fluorescence (ChlF), pigment ratios (Car Chl-a^{-1}, Chl-b Chl-a^{-1}), photosystem II (PSII) efficiency (F_v/F_m), and rapid light curve parameters (RLC).

Results revealed significant interactions between pesticide, light, and time, indicating that environmental conditions strongly modulate chemical stress. As hypothesized, the herbicide Basar caused the most persistent impairment of F_v/F_m and pigment homeostasis, particularly under strong light. Contrary to our expectations, the fungicide Ortiva also induced transient, partially reversible inhibition, whereas the insecticide Teppeki had comparatively moderate effects. OD generally reflected biomass trends but was occasionally decoupled from pigment-based measurements due to turbidity from Basar emulsions, highlighting the value of integrating ChlF for accurate biomass assessment. We detected species-specific responses: *S. leopoliensis* displayed dynamic photoprotective pigment modulation but was vulnerable to carotenoid disruption, while *C. vulgaris* experienced stronger suppression of PSII function yet retained the ability for pigment reallocation and partial recovery.

Overall, our findings demonstrate that pesticide toxicity in microalgae is context-dependent, influenced by both light intensity and exposure duration, and underscore the need to consider the group-specific metabolism when assessing ecological risk. Such insights are critical for understanding impacts on primary productivity, community composition, and aquatic ecosystem resilience.

Abstract

(Deutsch)

Pestizidabfluss stellt ein erhebliches Risiko für aquatische Primärproduzenten dar, doch die Effekte chemischen Stresses in Kombination mit der Lichtintensität auf Mikroalgen sind bislang unzureichend verstanden. In dieser Studie untersuchten wir die Auswirkungen dreier häufig eingesetzter Pestizide – des Herbizids Basar, des Fungizids Ortiva und des Insektizids Teppeki – auf Wachstum, Pigmentverteilung und photosynthetische Leistungsfähigkeit der Grünalge *Chlorella vulgaris* sowie des Cyanobakteriums *Synechococcus leopoliensis*. Basar enthält den Wirkstoff S-Metolachlor, Ortiva enthält Azoxystrobin und Teppeki enthält Flonicamid. Die Kulturen wurden sieben Tage lang unter schwacher und starker Bestrahlung mit stark verdünnten Pestizidlösungen exponiert. Physiologische Reaktionen wurden mittels optischer Dichte (OD), in vivo Chlorophyll-a-Fluoreszenz (ChlF), Pigmentverhältnissen (Car Chl-a^{-1}, Chl-b Chl-a^{-1}), Photosystem-II-(PSII)-Effizienz (F_v/F_m) sowie Parametern aus Rapid Light Curves (RLC) erfasst.

Die Ergebnisse zeigten signifikante Wechselwirkungen zwischen Pestizid, Licht und Zeit, was darauf hinweist, dass Umweltbedingungen den chemischen Stress maßgeblich modulieren. Wie erwartet verursachte das Herbizid Basar die stärkste und anhaltendste Beeinträchtigung von F_v/F_m und der Pigmenthomöostase, insbesondere unter hoher Lichtintensität. Entgegen unserer Erwartungen führte auch das Fungizid Ortiva zu einer vorübergehenden, teilweise reversiblen Hemmung, während das Insektizid Teppeki vergleichsweise moderate Effekte zeigte. Die optische Dichte spiegelte im Allgemeinen die Biomassetrends wider, war jedoch gelegentlich aufgrund von Trübungen durch Basar-Emulsionen von pigmentbasierten Messgrößen entkoppelt, was den Mehrwert der Integration von ChlF zur präzisen Biomasseabschätzung unterstreicht. Es wurden artspezifische Reaktionen festgestellt: *S. leopoliensis* zeigte eine dynamische photoprotektive Pigmentmodulation, war jedoch anfällig für Störungen der Carotinoide, während *C. vulgaris* eine stärkere Hemmung der PSII-Funktion aufwies, jedoch die Fähigkeit zur Pigmentumverteilung und teilweisen Erholung beibehielt.

Insgesamt zeigen unsere Ergebnisse, dass die Toxizität von Pestiziden in Mikroalgen kontextabhängig ist, durch Lichtintensität und Expositionsdauer beeinflusst wird und dass gruppenspezifische Stoffwechselunterschiede bei der Bewertung ökologischer Risiken berücksichtigt werden müssen. Diese Erkenntnisse sind entscheidend für das Verständnis der Auswirkungen auf Primärproduktion, Gemeinschaftszusammensetzung und die Resilienz aquatischer Ökosysteme.

1. Introduction

Over the past century, synthetic pesticides have been widely adopted to support modern agriculture and horticulture, providing effective protection of crops against insects, fungi, and weeds [1][2]. These chemicals are applied to maximize crop yield and quality while minimizing negative impacts on human health and the environment. Despite their advantages, the extensive and frequent application of pesticides has raised concerns about environmental persistence [3], non-target toxicity [4], and ecosystem disruption [5], highlighting the need for careful management and evaluation of pesticide use. Properly applied pesticides offer significant benefits for crop yield and protection. Persistent residues and runoff can cause unintended impacts, including toxicity to non-target organisms, broader ecological disruption [6], and inefficient market outcomes [7]. Public concern over the environmental impacts of agrochemicals has grown substantially in recent decades [8][9]. Widespread pesticide use in agriculture contributes to environmental contamination, primarily through surface runoff that distributes chemicals into soil, air, and water. Phase transfer processes and adsorption influence pesticide partitioning among environmental compartments–desorption dynamics [10][11][12]. Once absorbed by organisms, pesticides can accumulate in tissues, undergo partial or co-metabolic transformation into intermediate degradation products, or be fully mineralized [13].

Regulatory authorities monitor water bodies to assess chemical contamination and ensure compliance with water quality standards. Reports from the European Environment Agency (EEA) reveal that pesticide concentrations often surpass threshold limits in both surface waters and groundwater, highlighting ongoing inputs from agricultural sources and persistent stress on aquatic ecosystems [14][15]. For drinking water, permissible levels within the European Union are set at 0.1 $\mu g\ L^{-1}$ for individual pesticides and 0.5 $\mu g\ L^{-1}$ for the total concentration of all detected pesticides [16][17]. Legacy contaminants remain problematic despite legal controls. One prime example is atrazine. Following its ban in the European Union in 2007, which was based on evidence that its use could result in groundwater concentrations exceeding the EU's strict regulatory limits, the compound and its transformation products continue to be detected in groundwater at concentrations above threshold values, reflecting its high environmental persistence and mobility in soil–water systems [18][19][20]. Similarly, historically used insecticides such as dichlorodiphenyltrichloroethane (DDT) are characterized by pronounced environmental persistence and well-documented adverse effects on non-target organisms, including humans. Due to its high lipophilicity and resistance to degradation, DDT accumulates in sediments and biota, leading to long-term impacts.

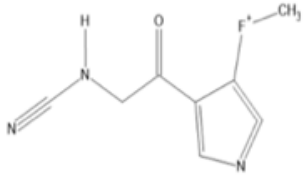
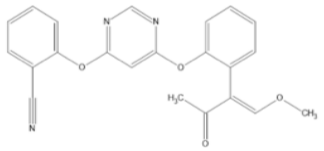
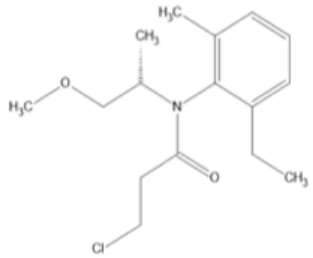
Among affected organisms, algae, as key primary producers within aquatic ecosystems, are particularly vulnerable to pesticide exposure, as contaminants can

interfere with photosynthetic processes [6][103], growth dynamics [6][110], and overall community structure [22][28]. Experimental studies have shown that *Chlorella vulgaris* exhibits concentration-dependent growth inhibition, impaired photosystem functionality, and altered physiological and biochemical parameters upon exposure to both legacy and currently applied pesticides [26]. The contamination of aquatic ecosystems by pesticides occurs through diverse environmental transport mechanisms, such as hydrological runoff from treated agricultural lands, atmospheric transport via spray drift and wind-driven dust particles [3][8], point-source inputs resulting from spills or improper handling, vertical leaching through soil matrices into groundwater reservoirs, and the release of inadequately removed residues via wastewater treatment plant effluents (WWTPs) [24]. Despite their fundamental role in protecting receiving water bodies, conventional WWTPs are not optimized for the efficient removal of trace organic micropollutants, including pesticides [27]. Their treatment configurations are primarily engineered to reduce bulk organic load, nutrients, and particulate matter, thereby allowing certain persistent and biologically active contaminants to pass through treatment processes and enter aquatic environments [28][29][30]. The ecotoxicological impacts of pesticides on algal communities can impair primary productivity and disrupt microbial consortia involved in biological treatment processes, thereby potentially reducing treatment efficiency and emphasizing the intrinsic link between aquatic ecosystem integrity and water quality management [13][24][31]. A comprehensive understanding of pesticide - algae interactions is therefore essential. Algae are sensitive bioindicators of ecosystem integrity, and their physiological and population-level responses to pesticide exposure provide critical insights into potential cascading effects within aquatic food webs [24][25][32]. Such knowledge provides a fundamental basis for ecological risk assessment, supports evidence-based regulatory decision-making, and contributes to the development of mitigation strategies to reduce pesticide contamination and safeguard aquatic biodiversity.

Given the ecological relevance of certain compounds released into the environment and their implications for aquatic ecosystem stability and water quality management, it is essential to evaluate their effects in a targeted manner. According to the diversity of pesticides, their types and classes, modes of action, and environmental behavior, their impacts on microalgal physiology and growth may differ substantially. In this study, we therefore focused on three pesticides Teppeki (insecticide), Ortiva (fungicide), and Basar (herbicide), which are used to fight different pest types.

1.1 Chemical Characteristics of the Investigated Pesticides

Table 1. The present study focuses on three commercial pesticide formulations: Teppeki (insecticide), Ortiva (fungicide), and Basar (herbicide).

Pesticide (CAS-Number)	Chemical Structure	Active ingredient	Chemical Formula	Active Ingredient Molecular weight	License holder / Supplier
Teppeki® 50 WG (158062-67-0)		Flonicamid	$C_9H_6F_3N_3O$	229.2 g·mol^{-1}	Ishihara Sangyo Kaisha Ltd.
Ortiva® (131860-33-8)		Azoxystrobin	$C_{22}H_{17}N_3O_5$	403.4 g·mol^{-1}	Syngenta Global AG
Basar® 960 EC (87392-12-9)		S-metolachlor	$C_{15}H_{22}ClNO_2$	283.8 g·mol^{-1}	Galenika-Fitofarmacija d.o.o.

1.1.1 Basar

S-metolachlor, sold under product names such as Basar, is widely used as a pre-emergence herbicide in maize and other crops to manage both annual grass and broadleaf weeds [33]. In contrast to atrazine, another widely used herbicide, S-metolachlor has recently undergone regulatory reassessment in the European Union. Based on concerns about groundwater contamination and potential risks to mammals, the European Commission decided not to renew the approval of S-metolachlor under the EU Plant Protection Products Regulation (EC No. 1107/2009), formally documented in Commission Implementing Regulation (EU) 2024/20. This means that products containing S-metolachlor will no longer be authorized for use in the EU, with Member States required to withdraw authorizations and ban its use, a decision driven by monitoring data showing that S-metolachlor and its metabolites can exceed drinking water limits and pose ecological hazards [18][31]. As one of the most widely used herbicides globally, S-metolachlor has been documented to influence non-target photosynthetic microorganisms [32][33][34]. In higher plants, its primary mode of action involves inhibition of cell division and elongation through interference with multiple enzymatic pathways, notably those associated with lipid biosynthesis and the production of very-long-chain fatty acids, which are essential for membrane formation and cellular growth [38][39][40]. These mechanisms suggest potential vulnerability of photosynthetic microalgae, such as *Chlorella vulgaris*, to S-metolachlor exposure, highlighting the ecological relevance of investigating its sub-lethal and physiological effects in aquatic environments [41]. In contrast, herbicides such as monuron act directly as Photosystem II (PSII) inhibitors, blocking electron transport within the photosynthetic apparatus [42][43]. Although these herbicides differ in their primary biochemical targets, both modes of action interfere with fundamental physiological processes conserved in photosynthetic organisms. These similarities suggest that essential metabolic pathways in microalgae may represent potential targets of herbicide toxicity. Consequently, toxic effects observed in algal species may reflect conserved or overlapping susceptibility mechanisms between vascular plants and microalgae [37].

Agricultural application of S-metolachlor results in its entry into surface water and groundwater, mainly through runoff and leaching after rainfall, as well as spray drift that carries the herbicide into nearby water bodies [44][45]. It is highly water soluble and moderately mobile in the environment [46][54][55], which facilitates its movement from soils into both surface water and groundwater systems, where it has been frequently detected (e.g., up to 37.9 μg L^{-1} in U.S. surface waters and several ~5.9 μg L^{-1} in groundwater samples) and can exceed regulatory thresholds for drinking water.

In response to environmental concerns, regulatory actions have been initiated in some regions to reduce the impact of S-metolachlor [18]. For example, the French Agency

for Food, Environmental and Occupational Health & Safety (ANSES) has moved to withdraw or restrict many uses of S-metolachlor due to concerns about groundwater contamination and risks to mammals, effectively banning its use [47]. These decisions reflect regulatory efforts to reduce environmental contamination. S-metolachlor, however, continues to be used in many non-EU countries, including the United States, Canada, Australia, China, and parts of Latin America, where it remains registered for weed control in multiple crops under regulated conditions, often with environmental hazard statements and risk mitigation measures to reduce runoff and environmental exposure [48].

The application of S-metolachlor is not recommended during rainfall events due to the increased risk of surface runoff and subsequent leaching into groundwater systems [49][50]. Precise precipitation prediction remains challenging in agricultural practice, complicating effective risk mitigation. The first rainfall event after S-metolachlor application has been shown to strongly influence export, with shorter intervals between application and rainfall resulting in greater runoff losses [51][52]. Agricultural runoff represents the primary pathway for S-metolachlor contamination of surface waters, as it may be transported either dissolved in water or adsorbed to eroded soil particles. Consistent with these properties, S-metolachlor and its transformation products have been detected in agricultural catchment water bodies [44][53].

Sorption and desorption processes further determine the environmental fate, mobility, and persistence of S-metolachlor in soils. Among soil properties, organic matter content plays a dominant role [11][53] in controlling sorption behavior. Several studies have demonstrated a strong positive correlation between organic matter content and the sorption coefficient (K_d), with higher organic matter levels resulting in substantially increased herbicide retention [57][58][59]. For example, soils with elevated organic matter content exhibited up to 6-fold greater sorption than soils with lower levels [58]. Because organic matter content decreases with soil depth, S-metolachlor adsorption is generally higher in topsoil than in subsoil layers [60][61]. While this enhanced retention in surface soils reduces vertical mobility, it may simultaneously increase persistence [62][63].

Due to the mobility of S-metolachlor and its metabolites, groundwater contamination has raised regulatory concerns [64][65][66]. In specific regions, such as designated protection zones (e.g., the Enns special protection area in Upper Austria), prior authorization is required for pesticide application to safeguard water resources. The Enns special protection area refers to the region surrounding the Enns River, a major watercourse in Upper Austria whose alluvial aquifers and associated groundwater bodies are important sources of drinking water. To protect these resources, Austrian water law establishes groundwater protection zones (Grundwasserschongebiete) in which pesticide use and other potentially contaminating activities are subject to

approval and additional restrictions [67]. Regulatory authorities initiated measures in 2023 to restrict the use of S-metolachlor-containing herbicides following the detection of S-metolachlor and its metabolites in groundwater at concentrations exceeding established water quality benchmarks [14][33]. Although groundwater monitoring has been conducted systematically since the early 20th century, harmonized European legislation establishing legally binding maximum permissible concentrations for pesticides and other physicochemical parameters was implemented only in 2001 [68].

Given the increasing environmental detection and regulatory attention surrounding S-metolachlor, understanding its effects on non-target aquatic organisms is of growing importance. Studies on green microalgae such as *Parachlorella kessleri* have shown that S-metolachlor exposure can promote oxidative stress, decrease growth, alter photosynthetic pigment concentrations, and affect cellular antioxidative responses even at concentrations much lower than classic toxicity thresholds, indicating that microalgae — a foundational component of aquatic food webs — are sensitive to environmentally relevant levels of this herbicide [35]. Although substantial progress has been made in advancing knowledge of algal biology, ecology, and physiology, research has predominantly focused on commercially relevant species such as *C. vulgaris* [69]. In contrast, comparatively less attention has been directed toward ecologically representative or non-commercial taxa, highlighting an important research gap [70]. Overall, S-metolachlor demonstrates moderate to extended persistence in soil environments, with the potential to impact groundwater and surface water quality [54].

1.1.2 Teppeki

Teppeki is an insecticide used to control aphids in various crops, including potatoes. It is highly toxic to bees and should not be applied to flowering plants while bees are foraging [71]. Teppeki's active ingredient is flonicamid. In contrast to many conventional insecticides, which are contact toxicants sprayed on plant surfaces, flonicamids are systemic [72], meaning they are absorbed by the plant and translocated throughout its tissues, making the entire plant lethal to the insects that feed on it. Aphids intoxicated with flonicamid exhibit significant behavioral alterations, including increased sensitivity to light, staggered walking with extended legs, altered righting reactions, and uncoordinated movement, which are distinct from behavioral effects caused by other insecticides [73]. Although flonicamid shows high efficacy against target pests, its chronic and acute toxicity to aquatic organisms is generally considered low. However, information on sublethal and community-level effects on aquatic invertebrates, particularly insects, remains limited, highlighting a significant knowledge gap in ecotoxicological research. For example, the green algae *Pseudokirchneriella subcapitata* exhibited the lowest toxicity response among tested trophic levels, with an EC_{50} (72 h growth inhibition) greater than 100 mg L^{-1}, indicating limited direct harmful effects at environmentally relevant concentrations

[74]. Studies have shown that flonicamid can enter surface waters and potentially groundwater via runoff, especially after rainfall or irrigation events following application. Concentrations detected in surface water tend to be low (typically <0.1 μg L^{-1}) [75], with no evidence that flonicamid promotes algal blooms [76]. Flonicamid has moderate persistence in soil, with a half-life of 6-19 days depending on soil type, suggesting a low but non-negligible risk of environmental exposure to non-target organisms [77].

In addition to algae, flonicamid is practically non-toxic to fish, birds, and earthworms at field-relevant concentrations. Still, it remains acutely toxic to honeybees if they forage on treated plants [71][72]. Therefore, while Teppeki poses minimal risk to most aquatic organisms and does not directly stimulate algal growth, careful application timing and adherence to label instructions are essential to protect pollinators and prevent runoff into sensitive water bodies [75]. Regulatory assessments, including the EPA memorandum supporting the proposed registration decision for flonicamid, indicate that no risks of concern were identified for aquatic invertebrates, with exposure estimates remaining below established levels of concern [78].

1.1.3 Ortiva

Ortiva 250 SC is a commercial formulation containing azoxystrobin [79]. Azoxystrobin is a widely used fungicide with broad-spectrum activity against fungal pathogens in a variety of crops, including vegetables, fruits, and ornamentals [80]. It exhibits systemic and translaminar properties, allowing uptake and redistribution within plant tissues, thereby providing both internal and external protection [81]. Due to its physicochemical properties and systemic behavior, azoxystrobin has the potential to enter surrounding environments through processes such as runoff and leaching [75]. The compound belongs to the strobilurin class of fungicides, which inhibit mitochondrial respiration in fungal cells by blocking electron transfer during oxidative phosphorylation, effectively preventing spore germination and fungal growth [82].

Reported environmental concentrations of azoxystrobin in an agricultural coastal watershed in the United States reached 4.6 μg L^{-1} , while concentrations in European streams have been observed as high as 30 μg L^{-1} [83]. These values indicate that azoxystrobin levels in surface waters can reach or exceed the effect concentrations identified for the test organisms in the present study. A study from Woodward et al. revealed [84] that although azoxystrobin was applied at a low dose (0.88 μg active ingredient m^{-2}), it exhibited the highest proportion loss to runoff, with 15.6 % of the applied mass transported away; however, the concentrations were below detection limits.

In terms of human health, azoxystrobin exhibits low acute toxicity. Still, it may pose significant risks if ingested at high doses, with potential effects on liver and thyroid functions observed in laboratory studies, leading environmental authorities to classify it as unlikely to be carcinogenic at environmentally relevant exposure levels [85]. Nevertheless, chronic exposure through contaminated water or food is carefully regulated through maximum residue limits (MRLs) in food commodities [86]. Environmental monitoring has detected azoxystrobin residues in surface waters adjacent to agricultural areas, and the compound has been found at low concentrations in groundwater in regions with intensive use and shallow water tables, indicating its potential to leach under certain soil and rainfall conditions [87][88][89]. Azoxystrobin is moderately persistent in soil, with reported half-lives ranging from approximately 7 to 30 days depending on temperature, moisture, and soil organic matter content, meaning it gradually decays through microbial degradation and chemical breakdown, but can remain bioavailable long enough to enter water bodies through runoff [90][91].

Although designed to control fungi, azoxystrobin can also affect non-target organisms. Eukaryotic algae, such as *Chlorella pyrenoidosa* [89], and cyanobacteria, such as *Synechococcus spp.* [92] have shown growth inhibition and reduced cell density at higher concentrations of azoxystrobin, indicating toxic effects beyond fungal targets. Fungicides containing azoxystrobin as the active ingredient have been associated with altered aquatic community composition, favoring cyanobacterial growth over sensitive algal species and potentially contributing to harmful algal blooms under certain conditions [93]. Widespread use of azoxystrobin has resulted in contamination of adjacent freshwater ecosystems, with reported concentrations ranging from 0.01 to 29.70 $\mu g\ L^{-1}$ in streams, ponds, groundwater, and lakes across Denmark [88], Germany [94], France [95], Brazil [96], and the USA [93].

Overall, while Ortiva is effective in controlling fungal diseases, its presence in the environment has implications for aquatic ecosystems, non-target algae, and water quality, warranting careful application timing, buffer zones near water bodies, and ongoing monitoring of surface and groundwater.

1.2 Study objective

The growth and behavior of numerous algal species are influenced by nutrient enrichment from human activities, such as agricultural runoff and wastewater discharge, which can alter community composition and sometimes promote harmful blooms [97][98]. However, the extent of these effects across different ecosystems and algal taxa remains poorly understood and is often overlooked in environmental risk assessments. The current study focuses on potential effects resulting from contamination by a herbicide, an insecticide, and a fungicide, all commonly used to control pests. In addition, we included a dilution series to determine the

concentrations at which these effects are strongest and at which pesticides have no discernible effect. We considered two strains of phototrophic organisms from different domains. *Chlorella vulgaris* SAG 211-11b is a single-celled Chlorophyceae (Eukarya), and *Synechococcus leopoliensis* SAG 1402-1 is a cyanobacterium (Bacteria). We mimicked pesticide washout concentrations typical of field applications. Test series were included to validate the lethal concentration of the respective pesticide to the target organisms. Moreover, the experimental design considered both strong and weak irradiance supply to test whether light intensity also influences the effects of pesticides. Specifically, we assessed the impact of pesticides on the growth, viability, and photosynthetic efficiency. The findings provide the basis for further research focusing on the factors that influence algal growth, the photosynthetic performance, biomass production, and potential alterations in cellular density of the species, thereby contributing to understanding the ecological risks posed by pesticide contamination in aquatic ecosystems. As prior research demonstrates, carry-over errors can produce unintended results [99]. Hence, the study was carefully managed through pilot experiments.

Based on the experimental design, we hypothesized that pesticide exposure would reduce growth, photosynthetic efficiency, and cell viability in both *C. vulgaris* and *S. leopoliensis*, with stronger effects expected at higher concentrations. We further anticipated that the two species would exhibit differential sensitivity due to their distinct cellular organization and domain-specific physiology, with the eukaryotic *C. vulgaris* potentially being more susceptible to some pesticides than the prokaryotic *S. leopoliensis*. In addition, light intensity is expected to modulate pesticide effects, potentially amplifying toxicity under high light conditions due to increased metabolic activity and reactive oxygen species (ROS) production, whereas low light may mitigate some impacts. Even sublethal concentrations may induce subtle changes in cell density, morphology, division rate, or photosynthetic efficiency, with broader ecological implications for natural aquatic communities. Together, these hypotheses and expectations guide the study's focus on understanding how pesticide contamination interacts with species-specific traits and environmental conditions to influence algal growth and ecosystem health.

2. Materials and Methods

2.1. Pilot experiment

The pilot experiment was designed to determine the maximum sublethal concentration of each pesticide, defined as the highest concentration at which the algae could continue to grow without experiencing mortality over a 7-day period. A small-scale 12-well microplate setup was employed (Costar® 12-well plates, Corning Life Sciences, Tewksbury, MA, USA; Cat. No. 3512), with *Chlorella*

vulgaris and *Synechococcus leopoliensis* serving as target organisms. Six replicates were prepared per pesticide concentration to ensure experimental reliability. In addition, controls without pesticide additions were cultivated. The target strains were obtained from the Culture Collection of Algae at the University of Göttingen (SAG, Göttingen, Germany): *C. vulgaris* SAG 211-11b (Chlorophyta) and *S. leopoliensis* SAG 1402-1 (Cyanoprokaryota). The strains were selected as representatives to evaluate the effects of pesticide exposure on both eukaryotic and prokaryotic photosynthetic microalgae. Each well had a working volume of 4 mL within a total well capacity of approximately 6.9 mL, allowing adequate gas exchange and minimizing the risk of spillage. Cultures were grown in BG-11 medium (Supplementary Table 1). The specific arrangement of concentrations within the costar plates is provided in *Supplementary Table 2*, with subsequent experiments following the same layout.

To establish a non-linear concentration–response profile for each pesticide, a dilution series was prepared at 25%, 10%, 5%, 1%, and 0.1% relative to the recommended field application rate. This approach ensured a broad concentration gradient and enabled the detection of both threshold and sub-lethal physiological and toxicological effects in microalgae. Although all three pesticides were tested across comparable relative concentrations (based on stock solutions) differences in their chemical composition and modes of action are expected to produce distinct toxicity profiles in microalgae. Teppeki, an insecticide targeting sap-feeding insects, may induce different physiological stress responses in algal cells compared to Ortiva, a fungicide, or Basar, a pre-emergence herbicide.

Stock solutions of each pesticide were prepared at their recommended field application concentrations, set at 100% in the experiment. For the stock solutions, Milli-Q water was used. Teppeki is typically applied at 1 $g \cdot L^{-1}$. For Ortiva, the recommended field application rate is 2 $mL \cdot L^{-1}$. (corresponding to a 500-fold dilution). The field application for Basar is 4 $mL \cdot L^{-1}$. In all cases, the final volume per well was 4 mL, with the pesticide volume complemented by medium and pre-culture to reach this total.

The corresponding volumes of each pesticide added to the wells are provided in detail in Supplementary Table 3. Volumes of added pesticides were in the µL range to minimize dilution effects of the culture medium. Pre-cultures were maintained for 2 weeks prior to experiments to ensure optimal physiological performance. Experiments were conducted at 21 °C under continuous low light (30 µmol photons m^{-2} s^{-1} photosynthetically active radiation) supplied by warm-white fluorescent lamps. Plates were placed on an orbital shaker (75 rpm) to maintain homogeneous suspension and promote algal growth.

Optical density (OD_{750}) and in vivo chlorophyll-a fluorescence (ChlF) were determined using a Tecan Spark® microplate reader (Tecan Group Ltd., Männedorf,

Switzerland). OD was measured at 750 nm, and ChlF was recorded in top-reading fluorescence mode (excitation: 410 nm; emission: 670 nm). In addition, Pulse-Amplitude Modulation (PAM) fluorescence was assessed to evaluate photosynthetic efficiency (F_v/F_m) during the pilot experiment using PAM-2500 (Heinz WALZ GmbH, Effeltrich, Germany). To minimize evaporation, plates were sealed with Parafilm immediately after each measurement. Before measurement, Parafilm was carefully removed, and samples were homogenized by repeated pipetting to ensure uniform cell suspension. Measurements were conducted sequentially, beginning with control wells and proceeding from the lowest to the highest concentrations to avoid cross-contamination. Pipette tips were replaced between treatments to maintain accuracy and reproducibility (Figure 1).

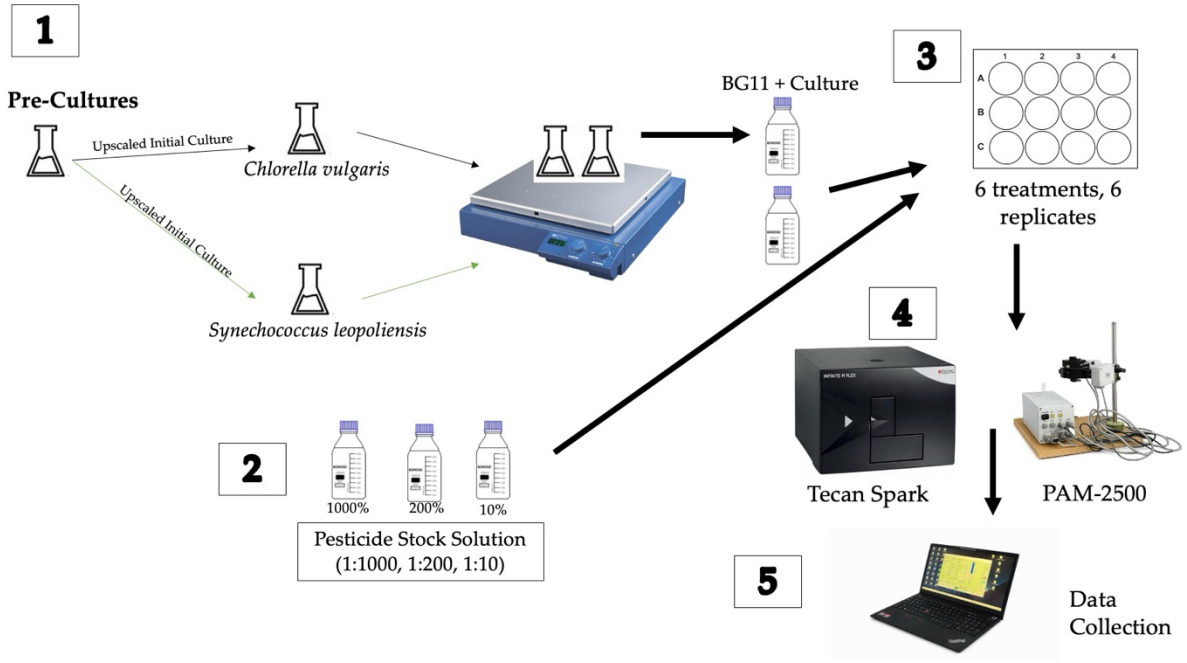


Figure 1. Pilot experimental workflow for assessing pesticide effects on *Chlorella vulgaris* and *Synechococcus leopoliensis*. Thick arrows indicate the main experimental steps, while thinner arrows represent preparatory or intermediate processes.

2.2 Main Experiment

For the main experiment, bubble column reactors were used as culture vessels due to several advantages: (1) they provide efficient homogenization of the algal dispersion compared to other reactor types, (2) they accommodate a wide range of culture volumes, including large-scale setups, and (3) they are straightforward to operate and maintain. A total of sixteen columns were employed per experimental run, with six runs planned overall, requiring approximately 96 liters of BG11 medium.

The experimental design included two microalgal species, *C. vulgaris* and *S. leopoliensis*, exposed to three pesticide treatments: Teppeki, Ortiva, and Basar. For each species, four replicates were established under the following conditions: (1) strong light (SL) with pesticide, (2) SL control (no pesticide), (3) weak light (WL) with pesticide, and (4) WL control (no pesticide). Light intensity levels were defined as SL = 70 μmol photons $m^{-2} s^{-1}$ and WL = 30 μmol $m^{-2} s^{-1}$ photosynthetically active radiation. This factorial design enabled the assessment of both individual and interactive effects of light intensity and pesticide exposure on algal growth dynamics. The pesticide concentrations used in the main experiment were selected based on the pilot experiment, with the concentration demonstrating the highest sublethal effect chosen for subsequent testing. This was 5% concentration of Teppeki and Ortiva, and a 1% concentration for Basar (see Supplementary Table 4). Continuous aeration with sterile air ensured adequate mixing and gas exchange, thereby maintaining homogenous culture conditions and supporting photosynthetic activity.

To evaluate the physiological and growth responses of the target organisms, multiple parameters were monitored throughout the experiment. These included OD and ChlF for estimating immediate biomass accumulation, dry mass (DM) for quantitative biomass assessment, PAM fluorescence after pre-darkening for maximum Photosystem II efficiency (F_v/F_m; overall photosynthetic performance), and rapid light curves (RLC) for photoadaptive responses, and spectrophotometric measurements of chlorophyll-a, chlorophyll-b, and carotenoids to characterize pigment composition during treatments.

2.3 Optical density (OD)

OD measurements were performed daily for 7 days using a VMR V-1200 spectrophotometer (VMR International, Radnor, PA, USA) at 750 nm. Prior to sample analysis, the instrument was calibrated by measuring the absorbance of the pure culture medium to establish a baseline (zero reference), followed by measurement of the algal replicates. Before transferring samples into the cuvette, each replicate was gently inverted to ensure homogenization of the suspension. Between samples, the cuvette was thoroughly rinsed with deionized water to eliminate residual material from previous readings. To ensure data accuracy, technical replicates were taken for each sample, and the averaged value was used for further calculations. Instrument calibration and maintenance were regularly performed according to the manufacturer's specifications to guarantee consistent performance throughout the study.

2.4 In vivo Chlorophyll a (ChlF)

ChlF was measured daily using a spectrofluorometer (SHIMADZU RF-6000; Shimadzu Corporation, Kyoto, Japan). Excitation and emission wavelengths were set

to 410 nm and 670 nm, respectively. Before measuring the algal samples, the instrument was calibrated using BG11 medium as a blank. To ensure homogenous suspensions, each replicate was gently shaken prior to cuvette filling. Fluorescence intensity values were recorded for each replicate and used as a proxy for algal biomass under in vivo conditions [100].

2.5 Pulse-Amplitude-Modulated (PAM) fluorescence

Photosynthetic performance was assessed by PAM-fluorescence of Photosystem II (PSII) at the initial (T_0) until the final (T_7) time point. PAM fluorescence provides a non-invasive and reliable approach for assessing photosynthetic efficiency and the physiological status of algal cells. Measurements were done daily using a PAM-2500 fluorometer (Heinz WALZ GmbH, Germany), specifically designed for low-density microalgal suspensions. Instrument settings, including measuring light intensity, gain, and damping, were adjusted as necessary to maintain the raw fluorescence signal within the range of 0.050–0.400.

F_v/F_m was daily monitored. Before measurements, samples were dark-adapted for 10 minutes to ensure full oxidation of the electron transport chain and to allow PSII reaction centers to return to their basal state. In the measurement routine, minimum fluorescence (F_0) is first measured in the dark-adapted state, representing fully oxidized PSII reaction centers. This is followed by maximum fluorescence (F_m) readings obtained following application of a saturating light pulse with all PSII reaction centers fully reduced. Variable fluorescence (F_V) was calculated as $F_m - F_0$, and the F_v/F_m was determined according to $F_v/F_m = (F_m - F_0) / F_m$ [101]. F_v/F_m values are presented in Supplementary Table 2. In vascular plants, maximum F_v/F_m values are typically around 0.83, reflecting optimal photosynthetic performance and the absence of physiological stress [102]. In contrast, cyanobacteria generally exhibit lower F_v/F_m values, ranging from approximately 0.4 to 0.6, due to the presence of phycobiliproteins that can interfere with chlorophyll fluorescence measurements [103].

In addition, RLCs were measured with the PAM fluorescence device on the initial day, as well as on the fourth and seventh day. These measurements provide insight into the photosynthetic responses of the algal cells during different treatments. From the RLCs, the effective quantum yields of photosystem II [Y(II)] at defined, increasing light steps were obtained, and values for the maximum electron transport rate (ETR_{max}), the initial slope of the curve (α), and the onset of saturation (I_k) were estimated. ETR_{max} estimates algal photosynthetic capacity under saturating light, while α reflects photosynthetic efficiency under low light intensities. I_k provides insights into the light acclimation properties. The parameters were calculated using the PAM Win-5 software (Version 5.21; Heinz Walz GmbH, Effeltrich, Germany) associated with the PAM fluorometer PAM-2500.

2.6 Dry mass (DM)

Dry mass measurements were conducted on the initial day, as well as on the fourth and seventh day of each experimental run. Rotilabo® glass fibre filters (Grade 393, Carl Roth GmbH + Co. KG, Karlsruhe, Germany) were utilized for filtration. Each filter was pre-combusted at 450°C and pre-weighed prior to sample filtration. After filtrating a defined volume of the suspension, the filter was dried for 24 hours in a drying cabinet at 92 °C before being re-weighed to determine the accumulated algal biomass. The difference of the empty filter and the dried filter plus material was taken to calculate the DM.

2.7 Chlorophyll-a, Chlorophyll-b, and Carotenoids

Algal samples were filtered onto glass fiber filters (Rotilabo® Grade 393, Carl Roth GmbH + Co. KG, Karlsruhe, Germany), which were then placed into pre-labeled aluminum foil. The filters were immediately frozen at −20 °C to promote cell burst. Subsequently, the frozen filters were cut into small pieces and submerged in 5 mL of 90% acetone. Pigment extraction was carried out using ultrasonic homogenization with a Branson Ultrasonic Sonifier 250 (Emerson Electric Co., St. Louis, Missouri, USA). The homogenized filters were stored in the dark at 4 °C for 12 hours. Following incubation, the samples were centrifuged at 4 °C for 10 minutes, and the resulting supernatant was carefully separated from the filter residue. Absorbance readings were obtained using a spectrophotometer at 480 nm, 630 nm, 647 nm, and 664 nm, as described by Lorenzen [104]. 90% acetone was set as a reference to zero.

2.8 Statistical Analysis

Statistical analyses were conducted using RStudio (R Core Team, 2023), with software JAMOVI V2.3.28.0 and SigmaPlot 14.5.

Differences in growth, ChlF, F_v/F_m, and cell density between treatments and over time were assessed using linear mixed-effects models (GLMMs) fitted with the lme4::lmer()function. The model included treatment (Treat_code), time (time), and their interaction as fixed effects, and replicate identity (Code) as a random effect:

y~1+Treat_code+time+Treat_code:time+(1|Code)

Residuals were assumed to follow a Gaussian distribution, and model parameters were estimated using the bobyqa optimizer with Satterthwaite's method for denominator degrees of freedom. Confidence intervals were computed using the Wald method, and model convergence was verified. For variables that did not meet

parametric assumptions, nonparametric Kruskal–Wallis tests were applied, followed by pairwise post-hoc comparisons using estimated marginal means (emmeans package) with Tukey adjustment for multiple comparisons. Normality and homogeneity of variance were assessed visually using histograms and quantile-quantile (Q-Q) plots, and residual plots were inspected to ensure model validity. Analyses were performed at a significance level of $\alpha = 0.05$.

3. Results

3.1 Visible Experimental Observations in the Laboratory

Pilot experiment

The pilot experiment revealed clear differences in the toxicity of the tested pesticides toward both *Chlorella vulgaris* and *Synechococcus leopoliensis*. Basar exhibited the strongest inhibitory effect, resulting in rapid loss of pigmentation and complete growth inhibition at concentrations ≥ 5%, indicating high toxicity in both species. In contrast, Ortiva showed moderate and species-specific effects: growth inhibition was observed at higher concentrations (≥ 10%), with *S. leopoliensis* responding more sensitively and with a delayed effect compared to *C. vulgaris*. Teppeki showed minimal to no inhibitory effects, with biomass development largely comparable to that of the controls across the tested concentrations.

These trends were supported by OD and ChlF measurements, although discrepancies between the two proxies were observed at higher concentrations due to increased turbidity caused by the formulations. F_v/F_m further confirmed the strong inhibitory effect of Basar and the moderate, concentration-dependent effects of Ortiva, while Teppeki had little impact.

Overall, the pilot experiment identified Basar as highly toxic, Ortiva as moderately inhibitory at higher concentrations, and Teppeki as largely non-inhibitory. These findings were used to define the concentration ranges and exposure conditions for the main experiment.

Main experiment

At the onset of the experiment, bubble column reactors showed transparent culture media with no visible coloration. We inoculated the same biomass amounts to different treatments to provide comparable starting conditions. By the end of the exposure period, pronounced treatment-dependent differences in pigmentation intensity were obvious (Figure 2).

For *C. vulgaris*, cultures exposed to Teppeki exhibited similarly dark green coloration under both strong light (SL) and weak light (WL), comparable to their controls, with no visible effect on biomass development. Under Ortiva, a light-dependent response was observed: under SL, pesticide-treated cultures (P) appeared lighter than the control (C), whereas under WL, treated cultures showed pigmentation intensity similar to the control. In contrast, Basar induced the strongest effect, with pesticide-treated cultures appearing markedly lighter under both light conditions, indicating reduced biomass or chlorophyll content. The corresponding controls, however, showed normal growth and dark green pigmentation under both SL and WL.

For *S. leopoliensis*, Teppeki-treated cultures showed pigmentation comparable to the control under both light conditions, suggesting no visible inhibitory effect. Under Ortiva, a slight reduction in pigmentation intensity was observed in treated cultures compared to controls, becoming more apparent over time but less pronounced than in *C. vulgaris*. In the case of Basar, treated cultures exhibited visibly reduced pigmentation compared to controls under both SL and WL. However, growth was still evident, indicating a sublethal effect rather than complete inhibition. Controls across all treatments consistently showed strong green pigmentation, reflecting normal growth.

Among the herbicide treatments, Basar induced the strongest visible sublethal effects. In *C. vulgaris*, growth was initially visible but declined over time, resulting in apparent mortality under both light conditions. In contrast, *S. leopoliensis* showed continued growth, although reduced compared to the control. Teppeki produced no visually detectable effects on either species, with growth patterns comparable to the control under both light regimes. Ortiva caused delayed and reduced growth in *C. vulgaris*, becoming apparent after approximately two days of exposure, while *S. leopoliensis* exhibited a similar but less pronounced reduction in growth starting around day four.

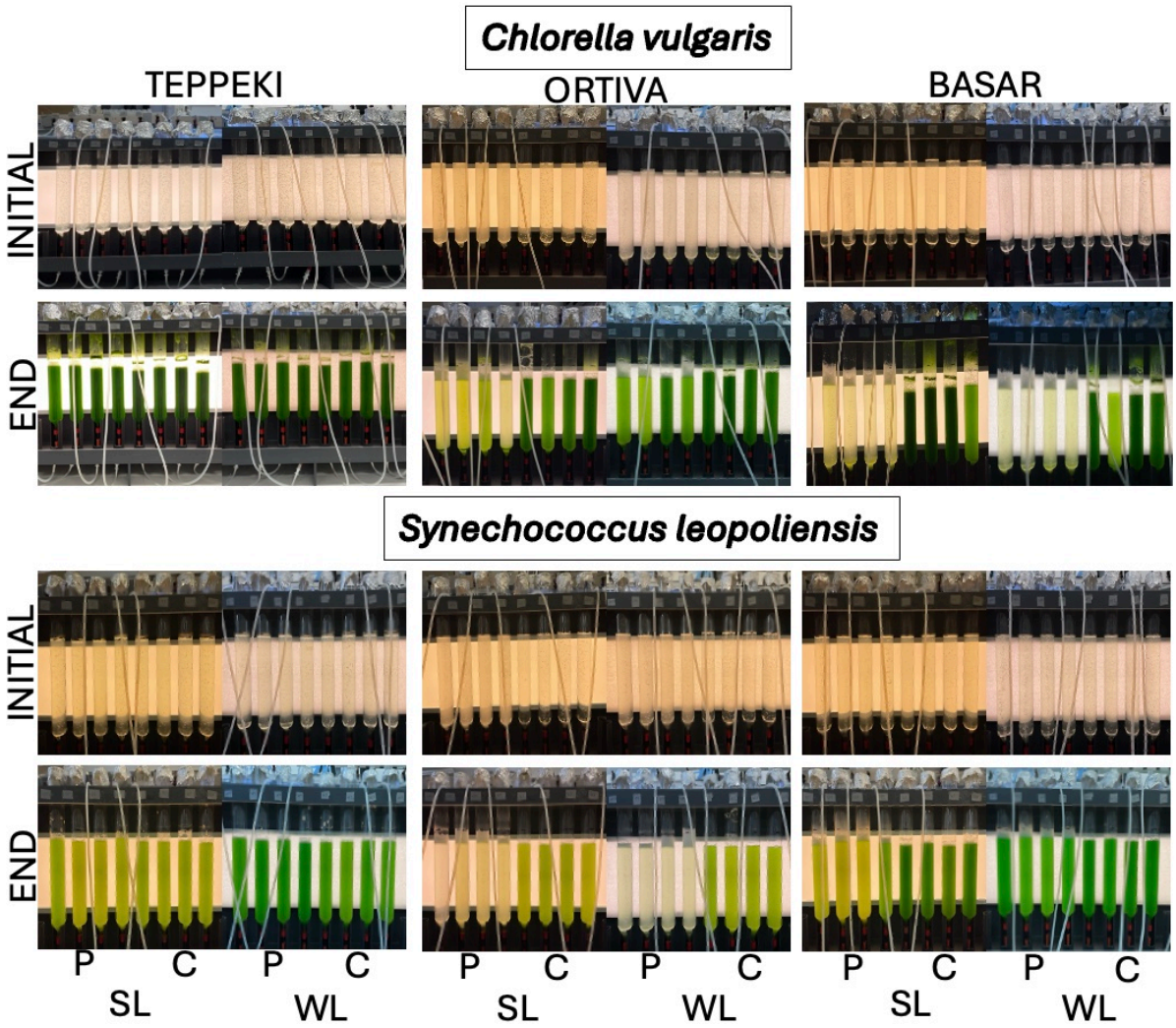


Figure 2. Development of *Chlorella vulgaris* **(top)** and *Synechococcus leopoliensis* **(bottom)** exposed to the herbicides. Bubble column reactors were set up under two light conditions: strong light **(SL)** and weak light **(WL)**. For each pesticide treatment **(P)**, four replicates were exposed to strong light and four replicates to weak light. Controls **(C)** are also included with four replicates under strong light and four replicates under weak light (right columns of the treatment), resulting in a total of 16 columns per run.

3.2 OD-ChlF as indirect estimators for algal biomass – a methodological comparison

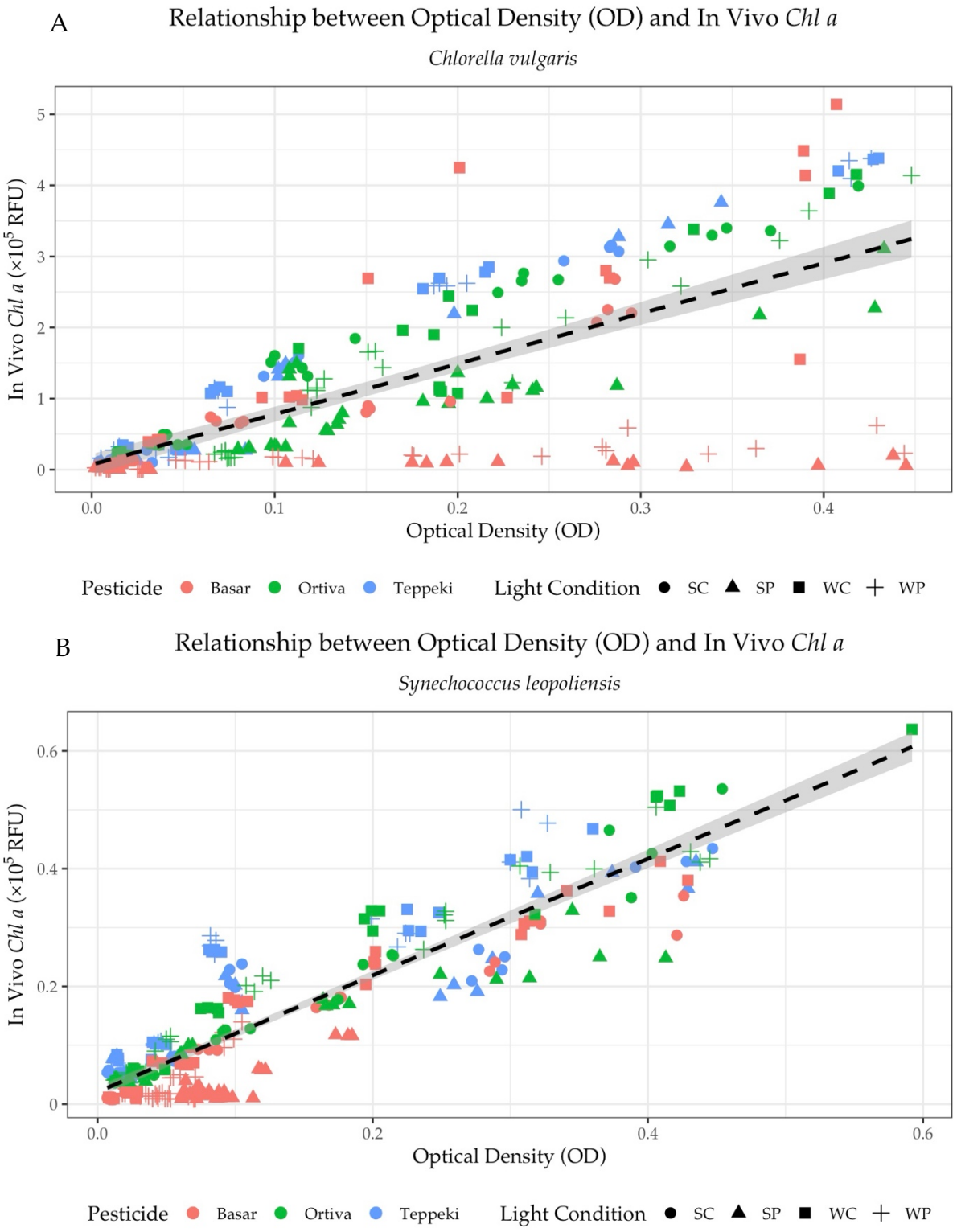


Figure 3. Relationship between optical density **(OD)** and *in vivo* chlorophyll *a* fluorescence **(ChlF)** in *Chlorella vulgaris* **(top)** and *Synechococcus leopoliensis* **(bottom)** across pesticide treatments and light conditions. Symbols represent individual observations, and lines indicate model-predicted trends with 95% confidence intervals. For visualization purposes, chlorophyll fluorescence values were expressed as ×10⁵ RFU; raw values were used for statistical analyses.

A significant, positive relationship between OD and ChlF was observed across all treatments and light conditions in *C. vulgaris* (Figure 3A). Overall, ChlF increased approximately linearly with increasing OD, as indicated by the fitted regression line and its confidence interval. However, substantial data scatter indicates considerable variability. At lower OD values (< 0.1), ChlF values were generally low (< 1×10^5 RFU) and relatively similar across treatments. As OD increased beyond ~0.1, divergence among treatments became more pronounced. Cultures exposed to Ortiva and Teppeki tended to exhibit higher ChlF fluorescence at comparable OD values, frequently exceeding 2×10^5 RFU at OD values between 0.2 and 0.4. In contrast, Basar-treated cultures showed greater variability, including several observations with comparatively low ChlF despite moderate to high OD values, which was caused by the turbidity of Basar in the first days of the experiments. Light conditions further contributed to the dispersion of data points. Under WL, ChlF fluorescence increased more steeply with OD than under SL. Pesticide treatments, specifically Basar, showed several data points with low fluorescence values even at high OD, as observed in the pilot experiment. This suggests that the differences in OD measurements were not directly indicative of biomass but were likely due to increased turbidity at higher Basar concentrations (a milky appearance).

In *S. leopoliensis*, a strong positive relationship between OD and ChlF was also evident (Figure 3B), with a more compact distribution of data points around the regression line compared to *C. vulgaris*. ChlF increased consistently with OD across the measured range (0–0.6), suggesting a tight coupling between cell density and pigment content; both parameters can be taken as reliable estimates for biomass development. At low OD values (< 0.1), ChlF remained below ~1×10^4 RFU for most treatments. As OD increased, ChlF values rose steadily, reaching approximately 4–6 × 10^4 RFU at OD values between 0.4 and 0.6. Ortiva- and Teppeki-treated cultures generally showed higher ChlF at a given OD compared to Basar-treated cultures, particularly at intermediate OD values (~0.2–0.4). Differences among light conditions were present but less pronounced than in *C. vulgaris*. WL cultures tended to cluster at the upper end of the ChlF range at a given OD, whereas SL cultures with pesticides showed slightly lower fluorescence. Overall variability was lower than in *C. vulgaris*, indicating a more uniform OD– ChlF relationship across treatments and light regimes in *S. leopoliensis*.

3.3 In vivo chlorophyll-a fluoresence (ChIF)

To investigate the interactive effects of pesticides and light on algal biomass, we used ChlF concentrations as a proxy for biomass (Figure 9). A GLMM was applied to disentangle the individual and interactive effects of treatments on biomass (Supplementary Tables 5 and 6).

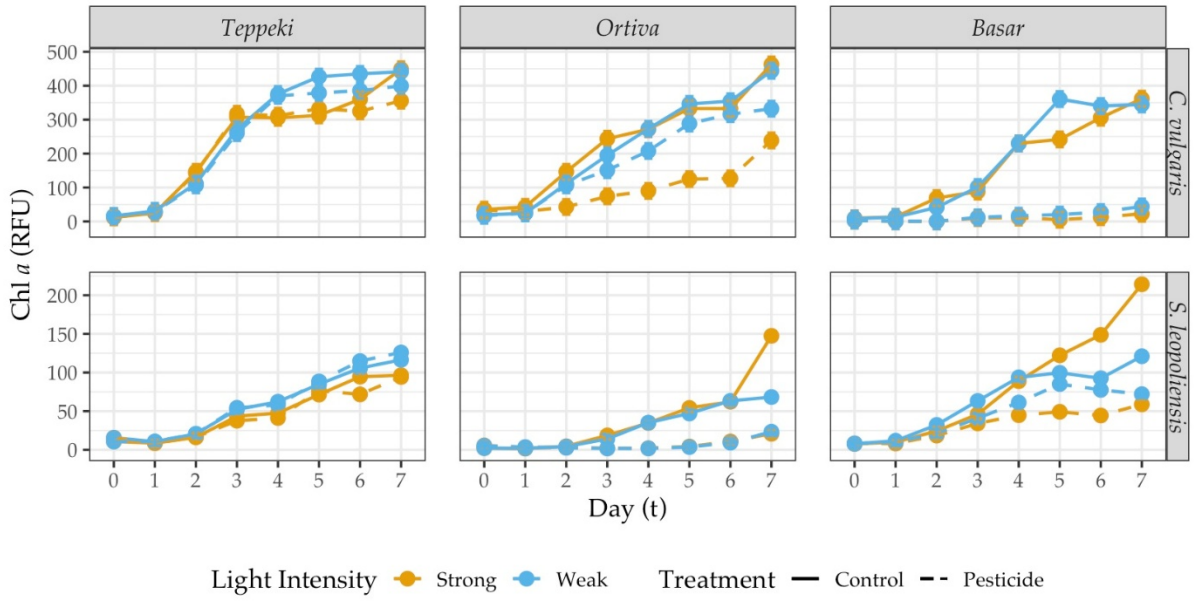


Figure 4. Effects of pesticide treatment and light exposure on in vivo chlorophyll *a* fluorescence **(ChlF)** in *Chlorella vulgaris* **(top)** and *Synechococcus leopoliensis* **(bottom)**. ChlF responses of *C. vulgaris* and *S. leopoliensis* under different pesticide treatments (Control, Teppeki, Ortiva, and Basar) and light conditions over the experimental period. Shown are temporal changes in ChlF under strong and weak light exposure. Data were analysed using generalized linear mixed-effects models to assess the effects of pesticide, light, day, and their interactions. Values represent modeled marginal means across sampling days (n = 4).

Overall, ChlF of ***C. vulgaris*** increased significantly over time, with large differences among pesticide treatments and light regimes. The GLMM revealed highly significant main effects of pesticide type, light, and day (all $p < 0.001$). In addition, significant interactions between pesticide × light, pesticide × day, and light × day (all $p < 0.001$) demonstrated that the effects of pesticide and light were not constant over time. Importantly, the three-way interaction between pesticide x light x day was also significant ($p < 0.001$), confirming that pesticide effects depended jointly on light intensity and exposure duration. Across all treatments, biomass increased markedly over time, particularly from day 2 onward, as reflected by the large and highly significant day effects in the model. Growth of the controls was maintained across days under all light conditions, reaching a maximum by day 7. In contrast, pesticide exposure altered both the magnitude and temporal dynamics of the response, with the extent of inhibition varying among compounds. Teppeki caused relatively moderate reductions, with treated cultures generally following trends of the controls, especially under WL. Ortiva induced stronger inhibitory responses that became apparent from day 3 onward, particularly under SL, where values were consistently lower than in the corresponding controls. Basar exerted the strongest inhibitory effect, markedly suppressing growth throughout the experiment, with treated cultures remaining low across all days and light treatments. Light conditions further

modulated the effects of pesticide exposure over time. Light-dependent divergence in ChlF patterns became increasingly evident at later time points. Under SL, pesticide-induced reductions were generally more pronounced, particularly for Ortiva and Basar, whereas under WL, it continued to grow even in the presence of pesticides. These patterns are consistent with the significant light × day and pesticide × light interactions identified by the model. The significant three-way interaction indicates that the combined influence of pesticide and light on algal biomass intensified with time, highlighting a dynamic response of *C. vulgaris* to prolonged chemical stress under different irradiance conditions.

ChlF of ***S. leopoliensis*** were strongly influenced by pesticide treatment, light conditions, and day, as demonstrated by the GLMM (pesticide($\chi^2 = 444.36$, $p < 0.001$; light $\chi^2 = 61.87$, $p < 0.001$; day $\chi^2 = 427.02$, $p < 0.001$). In addition, all interaction terms were significant ($p < 0.001$), indicating that temporal patterns differed depending on both pesticide exposure and irradiance conditions. Across all treatments, values increased significantly over time, with marked increases from day 3 onward relative to day 0 (day 3–7: all $p < 0.001$). The controls showed increasing ChlF throughout the experiment under all light conditions, reaching its highest values by day 7. In contrast, pesticide exposure significantly modified both the magnitude and temporal development of this increase. Teppeki caused comparatively mild effects, particularly under WL. Ortiva induced stronger reductions that became significant from mid-experiment onward, especially under SL. Basar produced the strongest suppression, with treated cultures remaining significantly lower than controls from day 3 onward across all light conditions, despite pronounced increases in the controls.

Light further modulated the effects of pesticides on growth, particularly under SL. This pattern is consistent with the significant light × day interaction and is reinforced by the significant three-way interaction, indicating that pesticide-induced suppression intensified with time and was most pronounced under SL.

3.4 Biomass-specific Chlorophyll-a contents

3.4.1 Chlorella vulgaris

Chl-a DM^{-1} dynamics in *C. vulgaris* were strongly influenced by both pesticide treatment and light intensity, with WL generally promoting higher pigment content except under persistent inhibition by Basar (Figure 5).

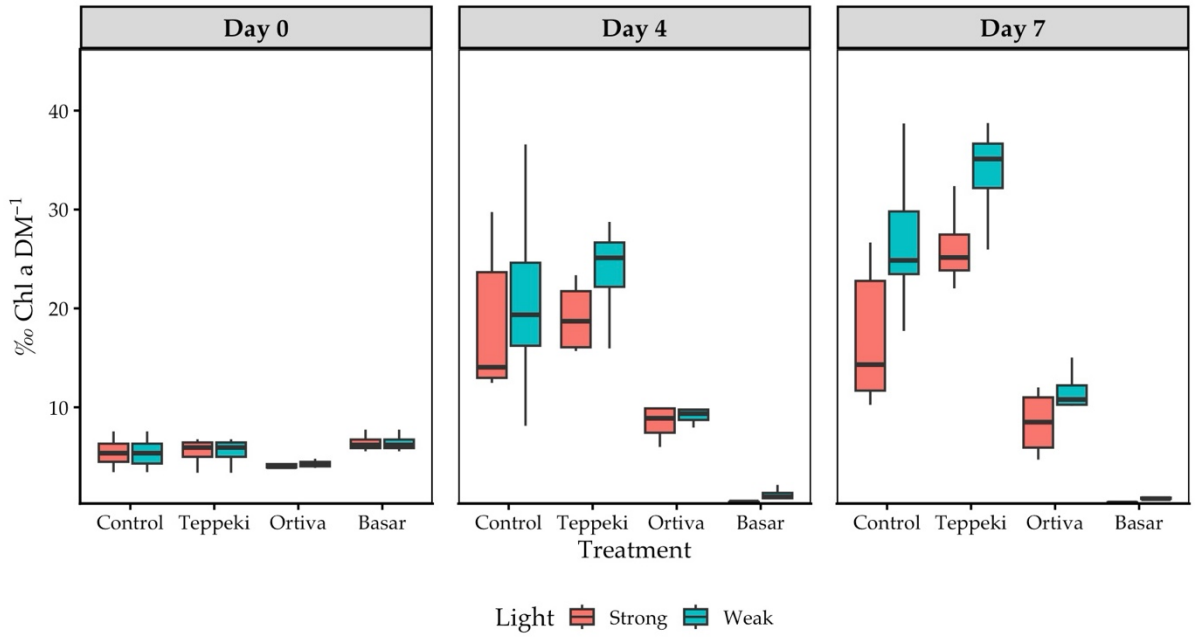


Figure 5. Chlorophyll-a content per dry mass of ***Chlorella vulgaris*** under different treatments over time. Chlorophyll-a per dry mass **(‰ Chl a DM⁻¹)** was measured in controls and cultures exposed to Teppeki, Ortiva, and Basar under strong and weak light on Day 0, Day 4, and Day 7. Boxplots show the median, the interquartile range, and the minimum and maximum values.

At Day 0, biomass-specific Chl-a concentrations were low and comparable across all treatments, ranging from approximately 3 to 6 ‰ Chl-a DM⁻¹. By Day 4, Chl-a DM⁻¹ increased significantly compared with Day 0, with an average increase of 12.7 ‰ Chl-a DM⁻¹ (± 2.1 SE, $p < 0.001$), reflecting active pigment synthesis during early growth. The response was strongly treatment-dependent: Basar significantly inhibited pigment accumulation, with values being approximately 18.7 ‰ lower than the corresponding control at day 4 (± 4.2 SE, $p < 0.001$) indicating a substantial suppression of pigment accumulation rather than an absolute decline below initial levels. Ortiva caused moderate reductions by −8.4 ‰ Chl-a DM⁻¹ (± 4.2 SE, $p = 0.046$) lower than the corresponding control at day 4, while control and Teppeki cultures showed substantial increases (~15–18 ‰ Chl-a DM⁻¹) under WL. Compared to SL, WL did not only influence chlorophyll-a levels but also altered their temporal development, resulting in a significantly greater increase by Day 7 (difference in change: +10.3 ‰ Chl-a DM⁻¹, ± 3.0 SE, $p = 0.001$). Elevated light intensities appeared to amplify pesticide-induced stress, potentially limiting pigment accumulation under SL. By Day 7, treatment- and light-dependent differences were even more pronounced: Teppeki under WL reached the highest specific Chl-a levels (~22 ‰ Chl-a DM⁻¹), the control remained moderate levels (~17–18 ‰ Chl-a DM⁻¹), Ortiva partially recovered (~13–15 ‰ Chl-a DM⁻¹), and Basar remained strongly inhibitory (~1–3 ‰ Chl-a DM⁻¹).

GLMMs confirmed significant treatment × day interactions, reflecting that treatment effects developed differently over time (Supplementary 7).

3.4.2 Synechococcus leopoliensis

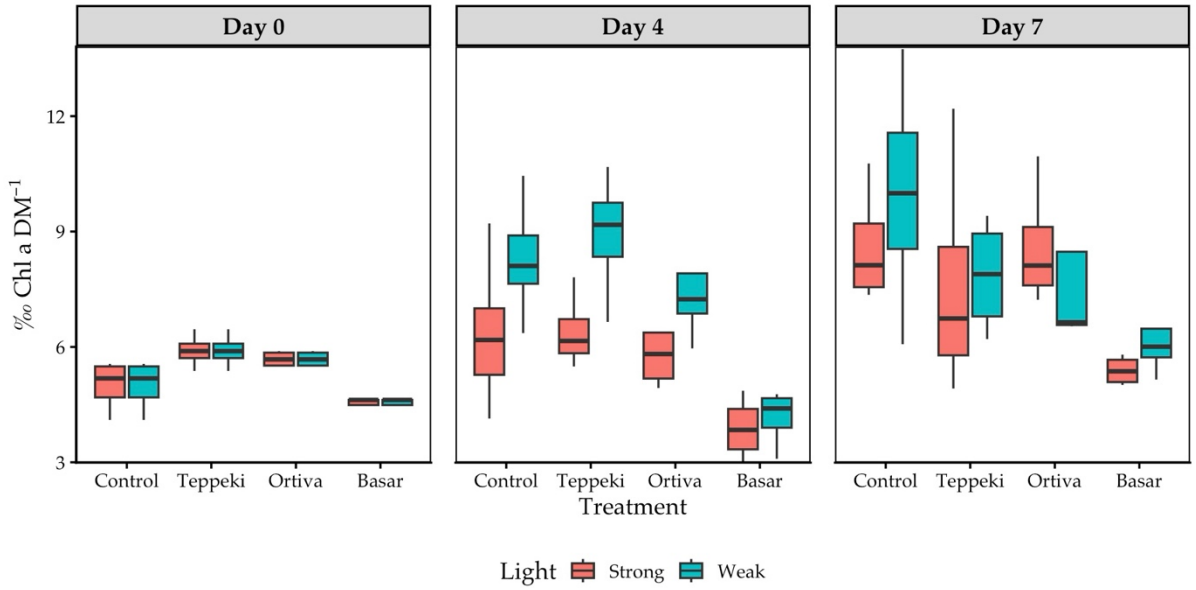


Figure 6. Chlorophyll *a* content per unit dry mass of ***Synechococcus leopoliensis*** under different treatments and light intensities over time. Chlorophyll *a* per dry mass **(‰ Chl-a DM^{-1})** was analysed in controls, Teppeki, Ortiva, and Basar under strong and weak light on days 0, 4, and 7. Boxplots show the median, the interquartile range, and the minimum and maximum values.

At day 0, specific Chl-a was low and similar across all treatments, ranging from approximately 4.5 to 6.0 ‰ Chl-a DM^{-1}, indicating comparable starting conditions, providing a consistent baseline for subsequent treatment effects. By day 4, biomass-specific pigment concentration increased significantly (+1.26 ± 0.63 ‰, p = 0.048), with a significant effect of light (WL vs SL: +2.10 ± 0.89 ‰, p = 0.021). Control and Teppeki showed a marked increase, particularly under WL, reaching median values of ~6–7 ‰, while Ortiva exhibited a moderate increase approximately ~5.5–6.0 ‰ at day 4. Basar remained the lowest among treatments (~4.5–5.0 ‰). Overall, WL promoted higher specific Chl-a concentrations than SL. At day 7, the values further increased (+2.83 ± 0.63 ‰ from day 0, p < 0.001), with WL resulting in a stronger increase (WL vs SL: +1.96 ± 0.89 ‰, p = 0.030). Controls reached the highest values (~7–8 ‰) under WL. Teppeki exhibited more variable responses, with ~6–7 ‰ under WL and slightly lower values under SL. Ortiva maintained moderate levels (~6 ‰), whereas Basar remained the lowest (~4.5–5 ‰).

GLMMs revealed significant effects of treatment ($F_{3,120}$ = 15.49, p < 0.001), light ($F_{1,120}$ = 7.50, p = 0.007), and day ($F_{2,120}$ = 23.94, p < 0.001) on Chl-a DM^{-1} in *S. leopoliensis*, indicating that both pesticide type and light intensity influenced pigment levels (Supplementary Table 8). In addition, a significant treatment × day interaction was detected ($F_{6,120}$ = 2.68, p = 0.018), demonstrating that the temporal development of chlorophyll a differed among treatments. In contrast, interactions between treatment and light, as well as the three-way interaction, were not statistically significant (p >

0.05). Across all treatments, WL consistently resulted in higher Chl-a DM^{-1} than SL, highlighting the influence of light intensity on photosynthetic pigment dynamics in *S. leopoliensis* (Figure 6).

3.5 Carotenoids per chlorophyll-a

We found a distinct difference between *C. vulgaris* and *S. leopoliensis* in their Car Chl-a^{-1} responses to pesticide treatments, light supply, and time. Across all treatments and sampling days, *S. leopoliensis* exhibited substantially higher Car Chl-a^{-1} ratios than *C. vulgaris*, indicating a greater baseline investment in accessory pigments. In *C. vulgaris*, Car Chl-a^{-1} values remained relatively low and changes over time were moderate, with only subtle differences between SL and WL. In contrast, *S. leopoliensis* showed pronounced light-dependent responses, with SL generally inducing higher Car Chl-a^{-1} ratios than WL (Figures 7 and 8).

Treatment-specific effects further emphasized species-specific sensitivity. In *C. vulgaris*, Basar consistently resulted in the highest Car Chl-a^{-1} ratios, especially under SL. Other treatments (Teppeki and Ortiva) induced only minor deviations from the controls. Conversely, *S. leopoliensis* displayed a strikingly different response to Basar, characterized by a strong reduction in Car Chl-a^{-1}, most notably at day 4 under both light conditions, with only limited recovery by day 7. Teppeki and Ortiva in *Synechococcus* showed clearer temporal trends than in *Chlorella*, including elevated Car Chl-a^{-1} under SL.

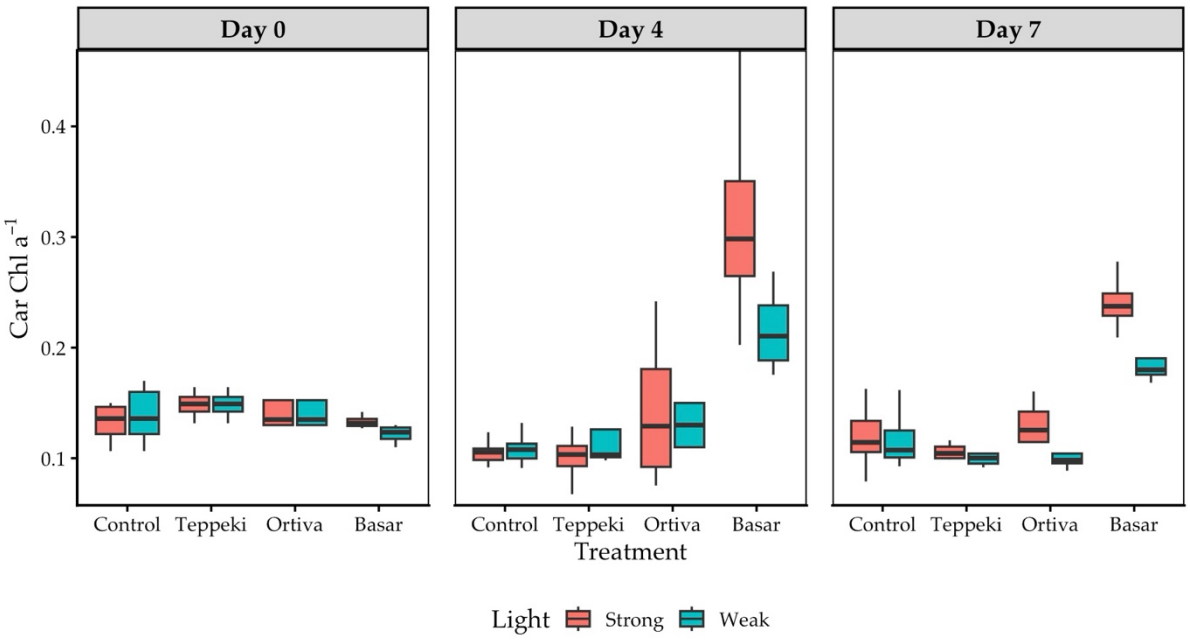


Figure 7. Species-specific effects of pesticide treatments and light regime on carotenoids per chlorophyll-a over time. Boxplots showing carotenoids per chlorophyll *a* ratios in *Chlorella vulgaris*

under control and pesticide treatments (Teppeki, Ortiva, Basar) exposed to strong and weak light at Days 0, 4, and 7. *C. vulgaris* shows lower values with Basar-induced increases consistent with photoprotective acclimation.

The effects of pesticide treatment, light intensity, and sampling day on the Car Chl-a^{-1} ratio of ***C. vulgaris*** were analyzed using a GLMM with replicate as a random effect (Supplementary Table 9). Treatment had a highly significant effect ($F_{3,120} = 35.57$, $p < 0.001$), while light intensity also influenced the ratio ($F_{1,120} = 5.26$, $p = 0.024$). Time had a marginal effect ($F_{2,120} = 2.97$, $p = 0.055$). Significant interactions were detected for treatment × light ($F_{3,120} = 5.29$, $p = 0.002$) and treatment × day ($F_{6,120} = 14.42$, $p < 0.001$), indicating that the impact of pesticides depends on both light conditions and time. The three-way interaction was not significant ($F_{6,120} = 1.24$, $p = 0.291$).

Post hoc pairwise comparisons of estimated marginal mean of *C. vulgaris* revealed that the Basar treatment strongly increased Car Chl-a^{-1} ratios relative to the controls (day 4 estimate = 0.212, t = 7.61, $p < 0.001$; day 7 estimate = 0.121, t = 4.35, $p < 0.001$) under SL. In contrast, Teppeki and Ortiva had smaller, often non-significant effects. Under WL, Basar still caused a significant increase on day 4 (estimate = –0.083, t = –2.27, $p = 0.025$), although the response was attenuated compared with SL. Treatment, light, and their interaction significantly affected Car Chl-a^{-1} ratios (Treatment: $F_{3,120} = 35.57$, $p < 0.001$; Light: $F_{1,120} = 5.26$, $p = 0.024$; Treatment × Light: $F_{3,120} = 5.29$, $p = 0.002$), whereas the three-way interaction was not significant (Treatment × Light × Day: $F_{6,120} = 1.24$, $p = 0.291$).

The random effect of replicates contributed negligibly to the total variation, as indicated by a near-zero variance estimate and by the model being close to singular, suggesting that almost all variation in Car Chl-a-1 ratios is explained by the fixed effects and residual error.

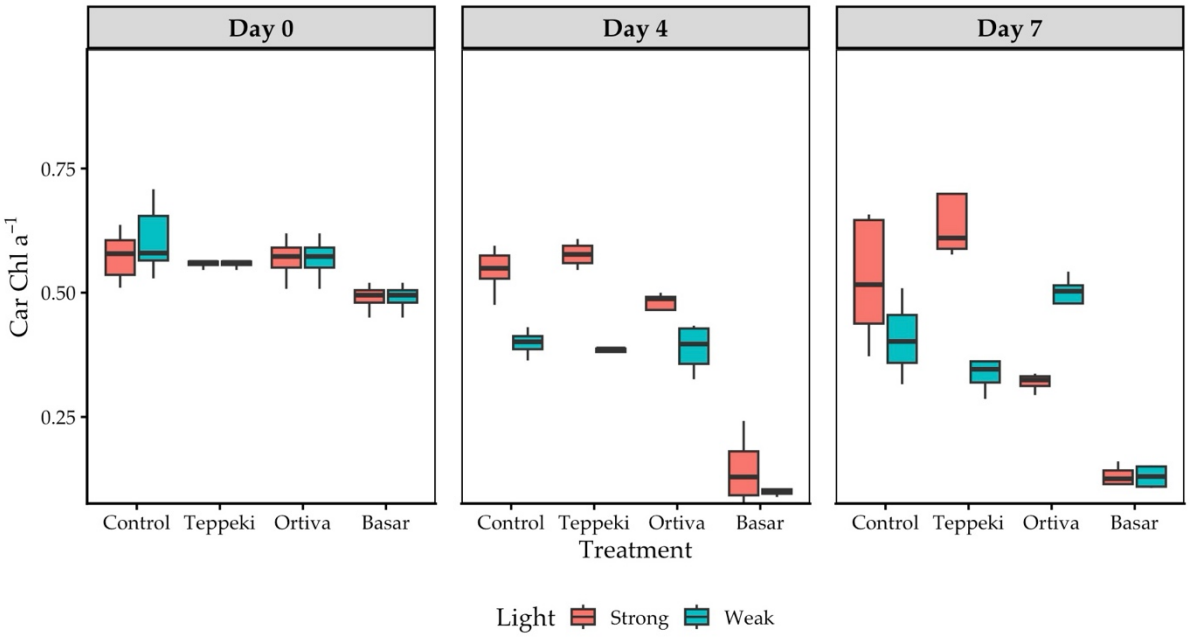


Figure 8. Species-specific effects of pesticide treatments and light regime on carotenoids per chlorophyll *a* (Car Chl-a^{-1}) over time. Boxplots showing Car Chl-a^{-1} ratios in *Synechococcus leopoliensis* under control and pesticide treatments (Teppeki, Ortiva, Basar) exposed to strong and weak light at Days 0, 4, and 7. *S. leopoliensis* displays higher overall Car Chl-a^{-1} ratios, stronger light-dependent responses, and a pronounced suppression under Basar.

The Car Chl-a^{-1} ratio on ***S. leopoliensis*** was significantly affected by pesticide treatment (F_3,27.2 = 57.71, $p < 0.001$), light intensity (F_1,12 = 10.88, $p = 0.006$), and day (F_2,108 = 55.86, $p < 0.001$), with significant two-way and three-way interactions between factors. Basar caused a strong reduction in Car Chl-a^{-1}, particularly on day 4 ($p < 0.001$) and Day 7 ($p = 0.0004$) under SL, while Teppeki and Ortiva showed smaller or variable effects depending on day and light. WL generally reduced the ratio compared with SL, especially on day 4 ($p = 0.0005$) and day 7 ($p = 0.01$). Three-way interactions further revealed that some pesticide effects were light- and day-dependent: for example, Teppeki under WL on Day 7 significantly reduced Car Chl-a^{-1} ($p = 0.041$), whereas Ortiva under WL on Day 7 increased the ratio ($p = 0.003$).

Estimated marginal means from the post hoc pairwise comparisons further highlighted these patterns (Supplementary 10): Basar consistently differed from the controls across days and light treatments, with the largest contrast observed on Day 7 under WL (estimate = 0.507, t = 9.71, p < 0.0001).

3.6 Chlorophyll-b per chloropyhll-a (Chl-b Chl-a^{-1})

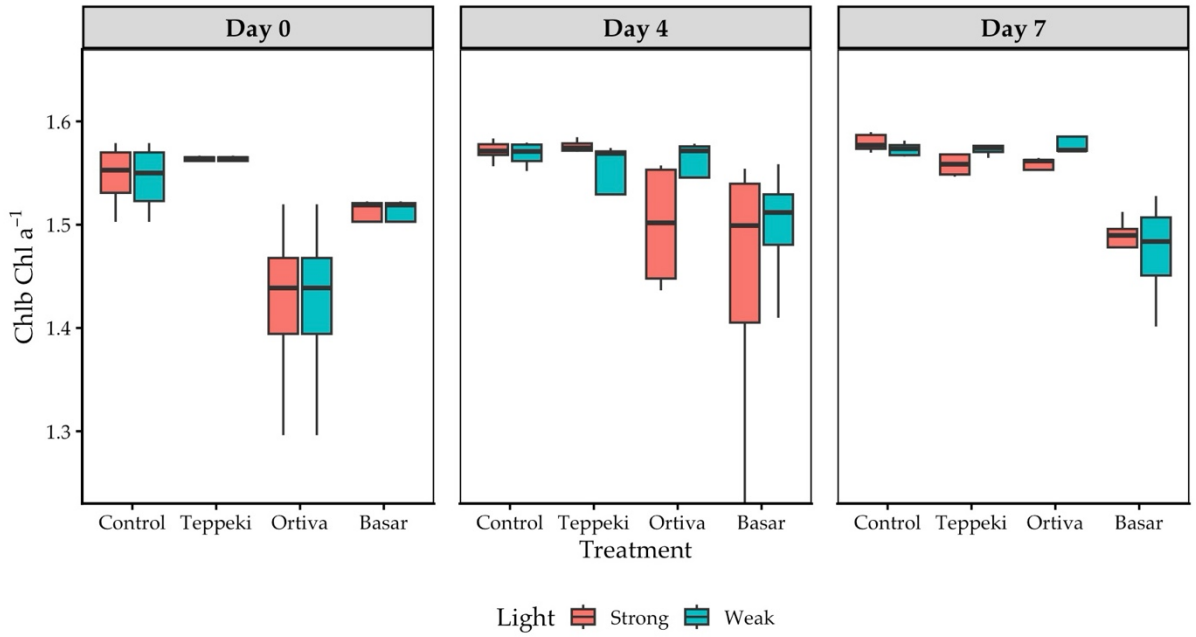


Figure 9. Chlorophyll a to chlorophyll b **(Chl-b Chl-a^{-1})** ratio of *Chlorella vulgaris* under control and pesticide treatments (Teppeki, Ortiva, Basar) exposed to strong and weak light at Days 0, 4, and 7. Data are shown as boxplots, with boxes indicating the interquartile range, horizontal lines representing medians, and whiskers indicating the range.

The Chl-b Chl-a^{-1} ratio of ***C. vulgaris*** varied across treatments, light conditions, and sampling days (Figure 8). The ratio was significantly influenced by treatment ($p = 5.11 \times 10^{-6}$) and sampling day ($p = 0.0185$), with a significant treatment × day interaction ($p = 4.21 \times 10^{-5}$), while light intensity and all light-related interactions were not significant. Overall, exposure to Ortiva resulted in a pronounced reduction in the Chl-b Chl-a^{-1} ratio compared with the control (−0.125 units, $p < 0.001$), indicating a strong treatment effect on pigment composition. This reduction was most evident at Day 0, where both control and Teppeki treatments exhibited significantly higher ratios than Ortiva under both light conditions (all $p < 0.05$), suggesting an immediate response following exposure.

Temporal patterns revealed distinct treatment-specific dynamics. Under Basar, the Chl-b Chl-a^{-1} ratio significantly decreased at day 4 (−0.082 units, $p = 0.0476$), indicating a delayed negative effect on pigment balance. In contrast, Ortiva showed a significant increase by day 7 relative to its initial effect (+0.108 units, $p = 0.0096$), suggesting partial recovery or acclimation over time. Post hoc comparisons further confirmed that Ortiva remained significantly lower at day 0 than the control and Teppeki at days 4 and 7 (all $p < 0.05$), as well as its own day 7 values under WL ($p \leq 0.005$), reinforcing the time-dependent nature of the response (Supplementary 11).

3.7 Pulse-Amplitude-Modulation (PAM) fluorescence

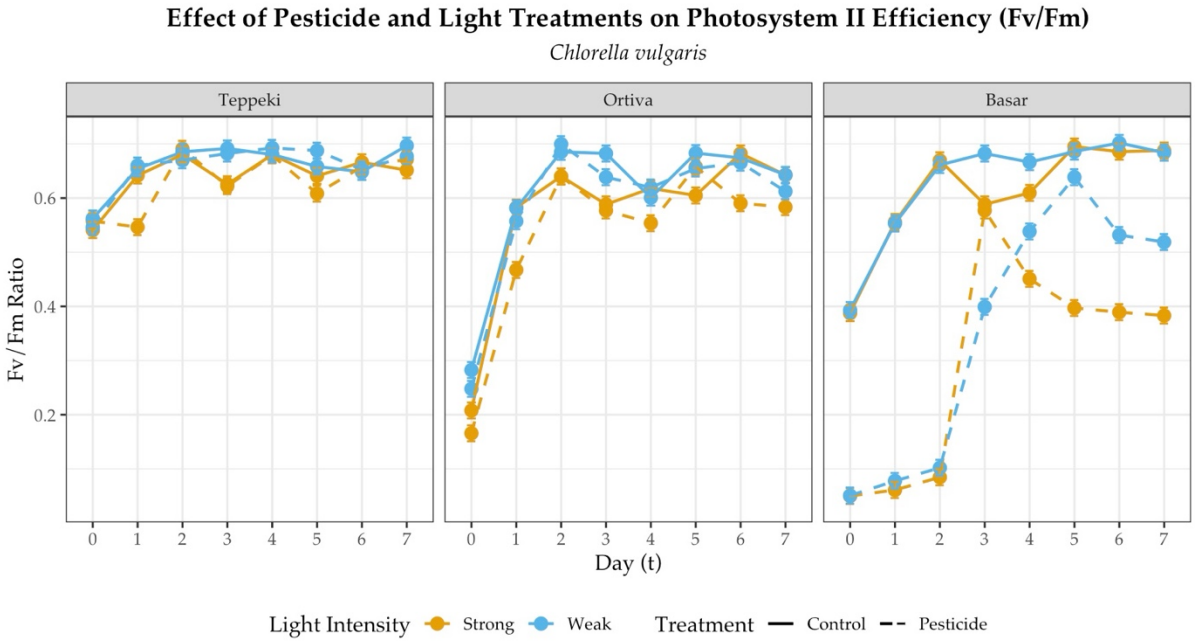


Figure 10. Photosystem II efficiency (F_v/F_m) of *Chlorella vulgaris* under pesticide and light treatments. Mean (± SE) photosystem II efficiency (F_v/F_m) of *C. vulgaris* measured over seven days under three pesticide treatments (Teppeki, Ortiva, and Basar) and two light intensities (weak and strong). Values are shown across sampling days to illustrate temporal responses and treatment-specific differences in PSII efficiency.

We observed significant interactions of F_v/F_m among fixed factors pesticide treatment, light supply, and time (all $p < 0.001$). In particular, the significant three-way interaction indicated that changes in PSII efficiency over time varied with both the applied pesticide and the provided light conditions on *C. vulgaris* (Supplementary Table 12).

In ***C. vulgaris***, day 0 under control light conditions, the mean F_v/F_m was 0.541 ± 0.015 SE. Relative to this reference, exposure to Ortiva significantly reduced F_v/F_m (model estimate = −0.333 ± 0.021, t = −16.0, $p < 2 \times 10^{-16}$), while Basar exposure also caused a significant decrease (model estimate = −0.153 ± 0.021, t = −7.36, $p = 1.89 \times 10^{-12}$). Light intensity alone did not significantly affect the response immediately after exposure (all contrasts $p > 0.30$). Across all sampling days, Teppeki-treated cultures exhibited relatively stable Fv/Fm values, generally ranging between approximately 0.60 and 0.70 under both WL and SL. Temporal effects were evident, with values increasing significantly over time relative to day 0 (day 1: +0.101 ± 0.021SE, $p = 2.11 \times 10^{-6}$; day 2: +0.140 ± 0.021SE, $p = 1.08 \times 10^{-10}$; day 7: +0.111 ± 0.021SE, $p = 2.20 \times 10^{-7}$).

Ortiva-treated cultures exhibited markedly lower F_v/F_m during the early phase of the experiment (days 0–1) at both light intensities. Significant pesticide × day interactions indicated a strong recovery over time for Ortiva (day 3 onward; model estimates and p-values). Under SL, Ortiva showed an additional but comparatively small reduction (model estimate = −0.059 ± 0.029, $p = 0.047$).

Basar treatment resulted in the strongest overall reduction in F_v/F_m. Under SL, values were significantly lower, with a substantial pesticide × light interaction (model estimate = −0.354 ± 0.029, $p < 2 \times 10^{-16}$). Although F_v/F_m increased over time under Basar exposure, the magnitude of recovery was smaller than that observed for Ortiva (day 3: +0.116 ± 0.029 SE, $p = 1.02 \times 10^{-4}$; day 6: +0.173 ± 0.029 SE, $p = 1.20 \times 10^{-8}$). Significant three-way interactions further indicated reduced PSII efficiency for Basar under both WL and SL at early and intermediate sampling days (e.g., Basar × SL × day 2: −0.241 ± 0.042, $p = 1.78 \times 10^{-8}$).

Post hoc comparisons of estimated marginal means (Bonferroni-adjusted) showed that on early sampling days, F_v/F_m under Ortiva and Basar was significantly lower than under Teppeki across both light intensities ($p < 0.0001$). At later sampling days, Ortiva did not consistently differ from Teppeki, whereas Basar remained significantly lower under SL at multiple time points (days 1–5; $p < 0.0001$). The random effect of replicate accounted for negligible variance (variance = 3.04×10^{-19}), indicating low variability among experimental replicates (Figure 10).

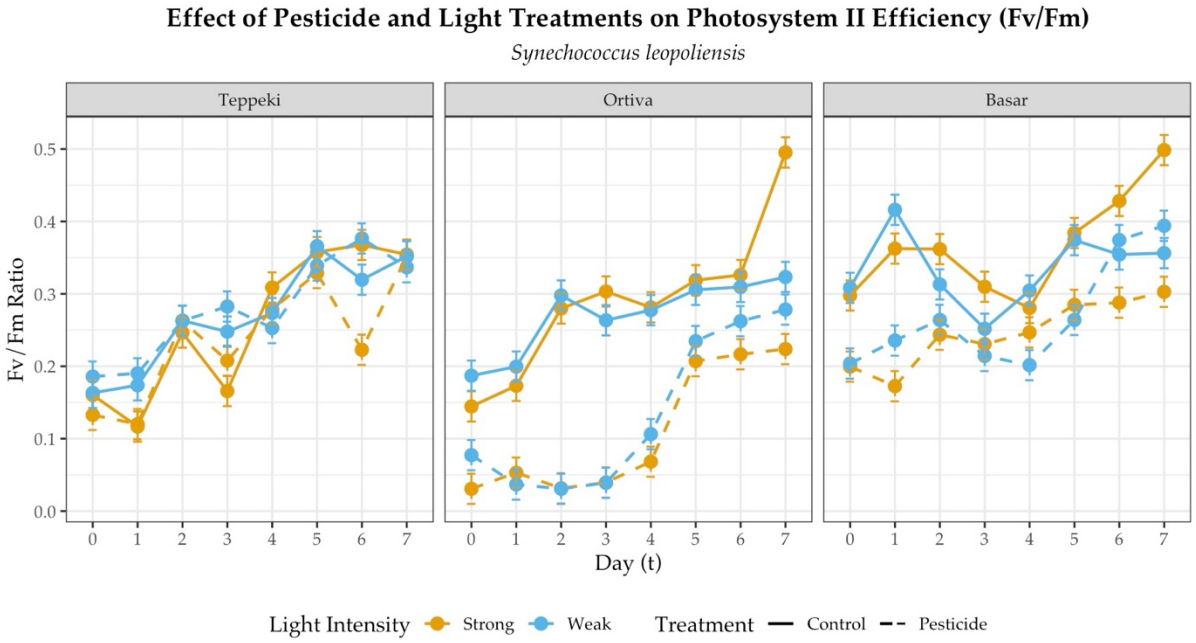


Figure 11. Photosystem II efficiency **(F_v/F_m)** of *Synechococcus leopoliensis* under pesticide and light treatments. Mean (± SE) PSII efficiency of *S. leopoliensis* measured over seven days under three pesticide treatments (Teppeki, Ortiva, and Basar) and two light intensities (weak and strong). Values are shown across sampling days to illustrate temporal responses and treatment-specific differences.

GLMM revealed consistent main effects on ***S. leopoliensis*** (Supplementary Table 13), as well as significant two-way and three-way interactions (all $p < 0.001$). The pronounced interaction ($F = 3.99$, $p < 0.001$) demonstrated that pesticide effects on F_v/F_m were strongly modulated by both light conditions and exposure duration. Across all treatments, F_v/F_m increased over time (day 2: $\beta = 0.086$, $p = 0.003$; day 4: $\beta = 0.149$, $p < 0.001$; day 7: $\beta = 0.194$, $p < 0.001$)

Basar exerted a strong overall effect compared to the control ($\beta = 0.138$, $p < 0.001$), whereas Ortiva showed no significant main effect when averaged across light conditions and time ($\beta = -0.016$, $p = 0.585$). Nevertheless, post hoc analyses revealed pronounced time- and light-dependent effects for Teppeki and Ortiva. Bonferroni-adjusted pairwise comparisons demonstrated that Basar significantly reduced values relative to the control at day 0 under SL conditions ($p = 0.0137$), with this difference persisting across subsequent days. Under WL and SL, Basar consistently resulted in lower values than the control at mid and late experimental stages (days 4–7; all $p < 0.01$). Ortiva exhibited a more variable response. While no consistent suppression was observed under WL, significant reductions in F_v/F_m occurred under SL and WL, particularly at days 2 and 3 (Ortiva × WL × day 3: $\beta = -0.288$, $p < 0.001$), indicating transient photoinhibitory effects that were partially alleviated at later time points.

Light supply significantly modified the effects of pesticides on PSII efficiency. Under SL, both Ortiva and Basar showed significantly lower values than the control at multiple time points (Basar × SL × day 1: $p = 0.035$; Ortiva × SL × day 2: $p = 0.002$). In contrast, WL generally mitigated pesticide-induced reductions in F_v/F_m, particularly for Ortiva. At later stages (days 6–7), several three-way interaction terms remained significant (Ortiva × WL × day 7: $\beta = -0.209$, $p < 0.001$; Basar × WL × day 7: $\beta = -0.147$, $p = 0.011$), confirming that light intensity and exposure duration jointly shaped the magnitude and persistence of pesticide stress.

Overall, *S. leopoliensis* exhibited a strong temporal increase in F_v/F_m across treatments; however, Basar caused sustained reductions in F_v/F_m, particularly under SL, whereas Ortiva induced transient, light-dependent effects that were most pronounced at early to mid-exposure periods (Figure 11).

3.8 Rapid Light Curves (RLC)

Rapid light curve parameters (α, ETR_{max}, and I_K) differed among pesticide treatments and light conditions in both *Chlorella vulgaris* and *Synechococcus leopoliensis* (Figures 12, 13).

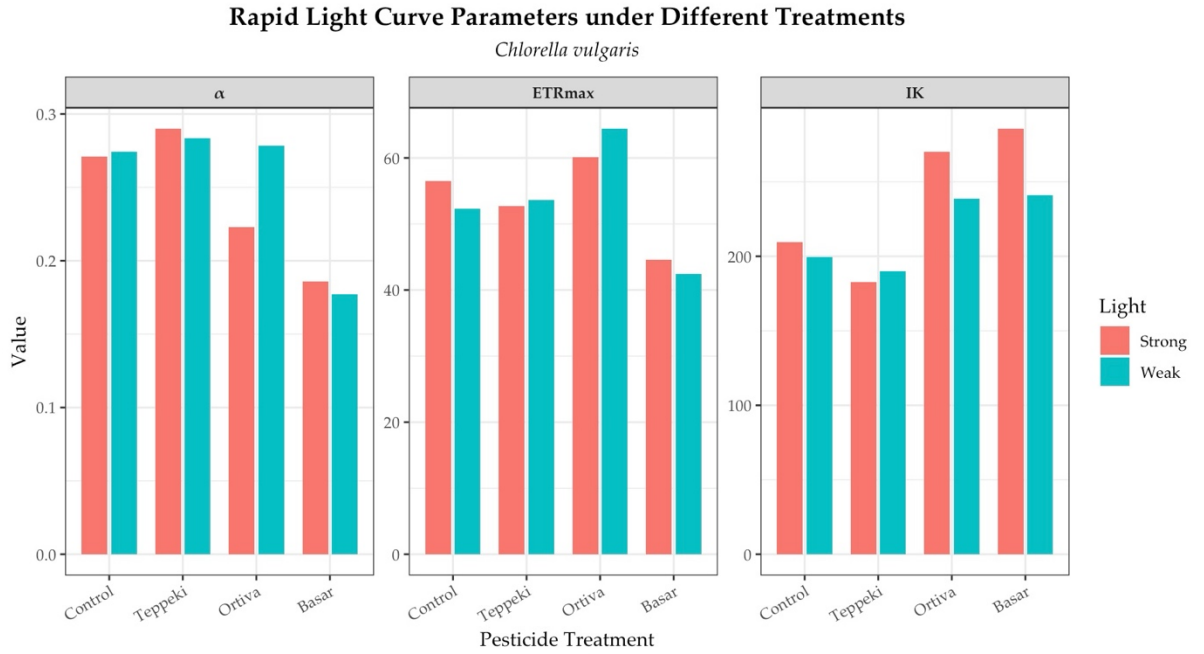


Figure 12. Rapid light curve **(RLC)** parameters of *Chlorella vulgaris* under different pesticide treatments and light conditions. Shown are the initial slope of the rapid light curve (α), the maximum electron transport rate (ETR_{max}), and the light saturation parameter (I_K). Bars represent mean values for each pesticide treatment (Control, Teppeki, Ortiva, and Basar) under strong and weak light conditions, with colours indicating light treatment. Values were derived from rapid light curves measured at the end of the experimental period.

In ***C. vulgaris***, α was highest in the control and Teppeki treatments under both light conditions, whereas reduced values were observed under Ortiva and Basar exposure, with the strongest reduction occurring under Basar. Differences between SL and WL were small for α, indicating limited light dependence for this parameter relative to pesticide effects. ETR_{max} were highest in the control and Ortiva treatments, particularly under WL, while Teppeki showed intermediate values and Basar consistently exhibited the lowest ETR_{max} under both light conditions. I_K increased under pesticide exposure, especially under Ortiva and Basar, with higher values under SL compared to WL, indicating photoacclimation to the respective light intensity.

Alpha (α)

The analysis revealed that pesticide treatment and time significantly affected α, whereas light quantity did not. GLMM showed a strong main effect of pesticide ($F_{3,120} = 49.93$, $p < 0.001$) and day ($F_{2,120} = 39.78$, $p < 0.001$), whereas light and all interaction terms were not significant. From the fixed effects, Ortiva ($p = 0.005$) and Basar ($p = 0.003$) significantly reduced α compared to the control, while Teppeki showed no significant effect. Additionally, α significantly increased over time, with both day 4 ($p < 0.001$) and day 7 ($p < 0.001$) showing higher values than day 0. Post hoc comparisons further supported these findings, indicating that temporal changes were consistently significant across treatments, whereas differences between pesticides

were less pronounced (Tukey adjustment of p values). Overall, these results suggest that α is strongly influenced by pesticide exposure—particularly by Ortiva and Basar—and increases over time but is not affected by light conditions.

Maximum Electron Transport Rate (ETR_{max})

For ETR_{max}, there were significant effects of pesticide, day, and their interaction, whereas light and its interactions were not significant. GLMM revealed significant main effects of pesticide ($F_{3,120} = 7.01$, $p < 0.001$) and day ($F_{2,120} = 69.08$, $p < 0.001$), as well as a significant pesticide × day interaction ($F_{6,120} = 8.21$, $p < 0.001$). Ortiva treatment led to significantly reduced ETR_{max} ($p = 0.005$), whereas Teppeki and Basar had no overall significant main effects. However, the significant interaction indicates that pesticide effects varied over time. Ortiva showed strong time-dependent effects, with significant increases at day 4 ($p = 0.019$) and day 7 ($p = 0.001$), while Basar significantly decreased ETR_{max} at day 4 ($p = 0.041$). Pairwise comparisons confirmed that ETR_{max} increased significantly over time (days 4 and 7 vs. day 0) across many treatments, especially under Ortiva exposure, where pronounced differences were observed. These findings indicate that ETR_{max} is strongly driven by temporal dynamics and that pesticide effects—especially Ortiva—are dependent on exposure duration.

Light Saturation Parameter (I_K)

The I_K parameter was significantly influenced by pesticide, day, and their interaction, with no significant effects of light. We found significant main effects of pesticide ($F_{3,120} = 10.71$, $p < 0.001$) and day ($F_{2,120} = 60.72$, $p < 0.001$), as well as a strong pesticide × day interaction ($F_{6,120} = 10.67$, $p < 0.001$). Ortiva significantly decreased I_K ($p = 0.039$), while Basar showed a marginal effect ($p = 0.060$). Over time, I_K increased significantly at days 4 ($p < 0.001$) and 7 ($p = 0.009$). The interaction effects revealed that pesticide impacts varied across time: Ortiva caused strong increases in I_K at Day 4 ($p < 0.001$) and Day 7 ($p < 0.001$), while Basar significantly decreased I_K at day 4 ($p = 0.027$) but increased it at day 7 ($p = 0.013$). Post hoc comparisons supported these results, showing significant differences primarily between day 0 and later time points, especially under Ortiva and Basar treatments. Overall, I_K responses indicate pronounced temporal shifts and treatment-specific dynamics, with pesticide effects becoming more evident over time (Supplementary Table 14).

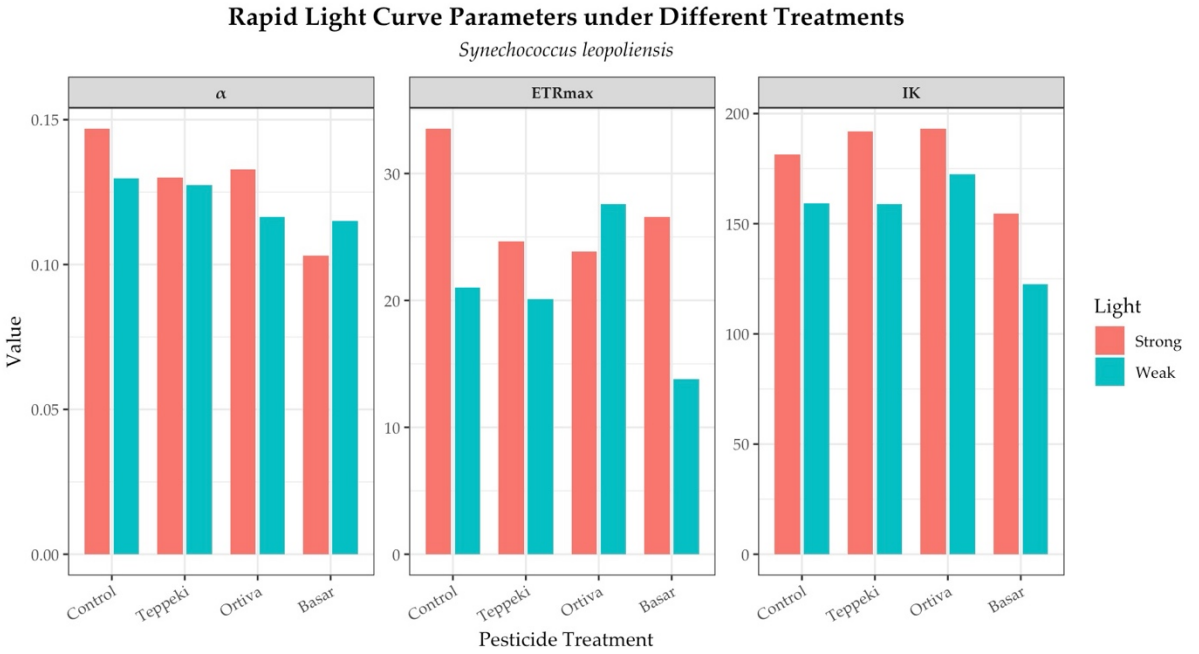


Figure 13. Rapid light curve **(RLC)** parameters of *Synechococcus leopoliensis* under different pesticide treatments and light conditions. Shown are the initial slope of the rapid light curve (α), the maximum electron transport rate (ETR_{max}), and the light saturation parameter (I_K). Bars represent mean values for each pesticide treatment (Control, Teppeki, Ortiva, and Basar) under strong and weak light conditions, with colours indicating light treatment. Values were derived from rapid light curves measured at the end of the experimental period.

The figure showed that pesticide exposure altered RLC characteristics in both species, with Basar exerting the strongest suppressive effects across parameters. Differences between strong and weak light were evident for ETR_{max} and I_K, whereas α appeared primarily driven by pesticide treatment rather than light condition. Species-specific responses were apparent, with *C. vulgaris* maintaining higher absolute values across parameters compared to *S. leopoliensis.*

In ***S. leopoliensis***, α values were generally lower than in *C. vulgaris* across all treatments and showed a consistent decline under pesticide exposure, with the lowest values observed under Basar. SL tended to yield slightly higher α than WL, although pesticide effects were more pronounced than light effects. ETR_{max} was highest in the control under SL and declined under pesticide exposure, with the most pronounced reduction under Basar, particularly under WL. Ortiva showed a relatively higher ETR_{max} under WL than under SL, indicating treatment-specific modulation by irradiance. The I_K parameter varied among treatments, with higher values generally observed under SL, indicating acclimation tot the provided irradiances. Basar consistently showed reduced I_K compared to the control and other pesticides, especially under WL.

Alpha(α)

Analysis of α in *S. leopoliensis* indicated strong main effects of pesticide ($F_{3,120} = 49.93$, $p < 0.001$) and day ($F_{2,120} = 39.78$, $p < 0.001$), while light and all interaction terms were non-significant. Examining the fixed effects showed that both the Ortiva ($p = 0.005$) and Basar ($p = 0.003$) treatments resulted in significantly reduced α compared to the control, whereas Teppeki did not. α increased significantly over time, with day 4 ($p < 0.001$) and day 7 ($p < 0.001$) showing higher values than day 0, indicating that photosynthetic efficiency improved as the culture matured. Post hoc comparisons further confirmed that temporal changes were consistently significant across treatments, while differences between pesticides were less consistently significant after Tukey adjustment. Overall, α in *Synechococcus* appears strongly modulated by pesticide exposure—particularly Ortiva and Basar—and shows a clear temporal increase, independent of light conditions.

Maximum Electron Transport Rate (ETR_{max})

ETR_{max} was significantly influenced by pesticide, day, and their interaction, while light and light-related interactions were not significant (pesticide $F_{3,120} = 7.01$, $p < 0.001$; day $F_{2,120} = 69.08$, $p < 0.001$; pesticide × day interaction $F_{6,120} = 8.21$, $p < 0.001$). Ortiva significantly reduced ETR_{max} overall ($p = 0.005$), while Teppeki and Basar did not show significant main effects. The significant interaction, however, indicated that pesticide effects were dependent on time. Ortiva exposure led to great temporal changes, with significant increases at days 4 ($p = 0.019$) and 7 ($p = 0.001$), whereas Basar caused a significant decrease at day 4 ($p = 0.041$). Pairwise comparisons confirmed that ETR_{max} generally increased over time across treatments, with the most pronounced changes under Ortiva. These findings suggest that temporal dynamics are the main driver of ETR_{max} responses in *Synechococcus*, and that the effect of pesticides, especially Ortiva, is modulated by exposure duration.

Light Saturation Parameter (I_K)

I_K was significantly affected by pesticide, day, and their interaction, with no significant effect of light (pesticide $F_{3,120} = 10.71$, $p < 0.001$; day $F_{2,120} = 60.72$, $p < 0.001$, pesticide × day interaction $F_{6,120} = 10.67$, $p < 0.001$). Ortiva significantly decreased I_K overall ($p = 0.039$), whereas Basar had a marginal effect ($p = 0.060$). Temporal effects were pronounced, with significant increases at days 4 ($p < 0.001$) and 7 ($p = 0.009$). The interaction revealed that pesticide impacts were time-dependent: Ortiva caused strong increases in I_K at days 4 ($p < 0.001$) and 7 ($p < 0.001$), while Basar decreased I_K at day 4 ($p = 0.027$) but increased it at day 7 ($p = 0.013$). Post hoc analyses confirmed these temporal and treatment-specific effects. Overall, I_K responses in *Synechococcus* indicate substantial temporal shifts, with pesticide effects becoming increasingly apparent over time (Supplementary Table 15).

4. Discussion

The effects of the tested pesticides varied markedly between species and treatments. Basar consistently induced pronounced responses, including reduced growth, altered chlorophyll fluorescence, and shifts in pigment ratios, reflecting its potent herbicidal action and direct interference with photosynthetic electron transport. In contrast, the fungicide Ortiva and insecticide Teppeki elicited comparatively subtle effects, causing only minor changes in ChlF and pigment composition. These differences likely stem from the compounds' modes of action: Basar directly targets biochemical pathways of photoautotrophs, whereas fungicides such as Ortiva primarily affect fungal metabolism and may only indirectly influence microalgal physiology at the applied concentrations. In contrast, Teppeki targets insect-specific physiological pathways that are not present in microalgae, which is consistent with its weak effects on the measured parameters. This pattern underscores the importance of considering pesticide type and mode of action when assessing ecological risks, as herbicides can produce more immediate and measurable impacts on microalgal growth and photophysiology than fungicides under similar exposure conditions.

We demonstrated that pesticide toxicity in *C. vulgaris* and *S. leopoliensis* is strongly modulated by light intensity and exposure duration. The consistent significance of pesticide × light × time interactions across multiple physiological parameters indicates that chemical stress [20] does not operate independently of the culture conditions provided. This supports growing evidence that herbicide toxicity in phytoplankton is influenced by light intensity rather than operating uniformly across light levels [105][22].

Based on pilot experiments, Teppeki and Ortiva were applied at a 5% working concentration, which produced measurable sublethal responses. Basar was administered at 1%, as this concentration was sufficient to induce sublethal effects in *C. vulgaris* while allowing limited but detectable growth in *S. leopoliensis*. These application levels align with pilot study outcomes, demonstrating consistent species-specific sensitivity patterns across the tested concentration range. Overall, light intensity was the primary determinant of biomass development, with pesticide exposure modulating species-specific growth responses. Variations in color intensity among pesticide treatments suggest compound-specific effects on algal growth, with SL conditions amplifying inhibitory effects, as visually evidenced by slower growth compared to WL treatments (Figure 2).

Optical density and in-vivo Chlorophyll as proxies for algal biomass

The positive relation between OD and ChlF observed in both species confirms that both parameters can serve as a proxy for biomass. OD is widely used as an indirect, rapid, non-destructive indicator of cell concentration, and its correlation with

chlorophyll content has been documented in both green algae and cyanobacteria under controlled growth conditions [106][107]. However, the reliability of OD as a biomass proxy depends on the stability of the culture medium's optical properties, cell size, age, physiological conditions, and bacterial contamination, all of which may be altered under chemical stress. A prime example of interference is Basar, which increased medium turbidity at high concentration, affecting OD measurements and decoupling turbidity-based estimates from pigment-based biomass monitoring. To avoid this potential interference, ChlF was chosen as the primary proxy for biomass. Thus, while OD remains a practical tool for rapid estimation of algal biomass, ChlF measurements provide a more robust and direct reflection of true biomass accumulation especially in turbid growth media.

Temporal Dynamics of In Vivo Chlorophyll a Fluorescence

Both *C. vulgaris* and *S. leopoliensis* showed that ChlF changed over time and was influenced by both light intensity and pesticide type. While ChlF in this study primarily served as an indirect proxy for biomass, rather than a measure of photosystem II (PSII) activity, it still provided useful insight into overall growth dynamics [105][22], in the context of this study it likely also captured changes in biomass, as increasing cell density contributes to higher bulk fluorescence signals. Therefore, the observed increases over time likely reflect biomass accumulation and increasing pigment content rather than growth alone.

In *C. vulgaris*, ChlF increased over time under control conditions, particularly under WL, consistent with sustained growth and/or increasing photosynthetic capacity. Under Basar exposure, ChlF remained low throughout the experiment, indicating strong suppression of physiological activity, likely involving both reduced growth and impaired photosynthetic function. Ortiva-treated cultures showed smaller increases in ChlF compared to the control, suggesting a progressive limitation of physiological performance over time rather than an immediate toxic effect. Under SL, these differences became apparent earlier, indicating that higher irradiance amplified the impact of pesticide exposure. Comparable patterns were observed in *S. leopoliensis*, although responses were slightly delayed. ChlF increased steadily in control and Teppeki treatments, indicating sustained growth. In contrast, Basar exposure resulted in consistently lower ChlF values, reflecting reduced performance. Under Ortiva, declines in ChlF became more apparent at later time points, suggesting a delayed treatment effect. Teppeki did not induce visible reductions in ChlF in either species. Instead, fluorescence increased over time in both *C. vulgaris* and *S. leopoliensis*, with patterns largely comparable to their respective controls under both light conditions, indicating no clear inhibitory effect on PSII-related activity or overall physiological performance within the observed time frame.

Light intensity further modulated pigment-related responses. In *C. vulgaris*, lower ChlF under SL coincided with higher carotenoid-to-chlorophyll ratios, suggesting

increased photoprotective investment [125]. In *S. leopoliensis*, ChlF suppression under Basar occurred alongside relatively stable, high carotenoid levels, indicating a distinct acclimation pattern. In contrast, *C. vulgaris* showed comparatively stable pigment ratios, consistent with different photoprotective strategies between taxa. This difference may be linked to the presence of the xanthophyll cycle in green algae [106], which enables dynamic regulation of excess excitation energy, whereas cyanobacteria rely on alternative carotenoid-based protective mechanisms. Overall, temporal ChlF patterns suggest that both species initially adjusted to environmental conditions but experienced cumulative physiological constraints under sustained pesticide exposure, particularly under SL.

Chlorophyll-a Allocation per Unit Biomass under Pesticide and Light Stress

Chl-a DM^{-1} clearly revealed species-specific and biomass-specific physiological adjustments to combined pesticide and light treatment. Cellular Chl-a allows to detect changes in pigment allocation and photosynthetic investment per unit biomass [109][110][111]. In *C. vulgaris*, Basar exposure caused a sustained reduction of Chl-a DM^{-1}, reflecting impaired pigment biosynthesis and/or enhanced degradation, potentially associated with oxidative stress and disrupted electron transport mechanisms [23]. This pattern is consistent with previous findings from pesticide experiments, in which Basar exerted pronounced inhibitory effects on photosynthetic performance and pigment status [112]. Differential sensitivity of green algae and cyanobacteria to pesticides has been widely documented [3], with green algal taxa often showing greater vulnerability to many chemical stressors than cyanobacteria [113]. These group-level differences are interpreted as arising from intrinsic physiological and structural variability — for example, differences in cell wall composition, membrane properties, and the diversity of detoxification and antioxidant systems — which influence uptake, target-site sensitivity, and the capacity to mitigate oxidative and cellular stress [114].

Light intensity significantly modulated these responses. Under WL, the Chl-a DM^{-1} ratio was generally higher, reflecting typical photoacclimation strategies in which microalgae increase pigment density to maximize light harvesting [115][116]. In contrast, SL reduced chlorophyll allocation per biomass, particularly under pesticide exposure, suggesting that combined photoinhibitory and chemical stress limited the capacity for pigment maintenance. Elevated light levels have been shown to enhance the toxicity of PSII-inhibiting herbicides, such as atrazine and simazine, in laboratory experiment with cultured freshwater phytoplankton by increasing metabolic demand and oxidative pressure, with stronger inhibitory effects on growth and photosynthesis under different light regimes [117]. Species-specific differences further indicate intrinsic physiological variability in pigment regulation, detoxification capacity, and stress tolerance mechanisms [118][119]. Such variability determines whether chlorophyll investment per unit biomass is maintained, reduced, or temporarily

compensated under combined stress conditions, a phenomenon documented across diverse microalgal taxa [120].

Overall, obtained Chl-a DM^{-1} ratios suggest that pesticide impacts extend beyond growth inhibition and involve alterations in cellular resource allocation and photoacclimation capacity. These interactive effects between chemical exposure and light conditions are critical for understanding how pesticide washout may influence primary productivity and energy flow in aquatic ecosystems. The higher pigment plasticity observed in *S. leopoliensis* suggests dynamic acclimation capacity, whereas stronger suppression in *C. vulgaris* under certain treatments indicates taxon-specific vulnerability. Such variability complicates generalized predictions of pesticide effects at the community level.

Accessory Pigment Allocation

The Car Chl-a^{-1} ratio provides a general indication of accessory pigment composition and potential acclimation responses under varying light and stress conditions. Cyanobacteria generally maintain higher carotenoid proportions, reflecting their reliance on accessory pigments for energy dissipation, ROS scavenging, and quenching of excess excitation [121][122]. In this study, consistently elevated Car Chl-a^{-1} ratios in *S. leopoliensis* suggest acclimation to irradiance conditions compared to *C. vulgaris*. Structural and functional differences in antenna organization between cyanobacteria and chlorophytes further contribute to distinct pigment regulation strategies [123]. Under SL, increased Car Chl-a^{-1} ratios in *S. leopoliensis* indicate dynamic adjustment of accessory pigments to mitigate light stress [124][125], whereas comparatively stable ratios in *C. vulgaris* point to alternative acclimation mechanisms, such as xanthophyll cycling or efficient photochemical regulation [105]. Stability in the ratio suggests maintenance of pigment homeostasis despite external stressors. Pesticide exposure elicited species-specific responses. In *C. vulgaris*, increased Car Chl-a^{-1} ratios under Basar indicate retained capacity to adjust accessory pigments under combined light and chemical stress [3]. Additionally, Basar consistently resulted in the highest Car Chl-a^{-1} ratios, suggesting an alteration in pigment composition. This may indicate enhanced Car Chl-a^{-1} ratios as a photoprotective response, but could also result from a decline in chlorophyll a (e.g., bleaching), highlighting the need to consider absolute pigment concentrations. In contrast, *S. leopoliensis* showed a decline in Car Chl-a^{-1} ratios, suggesting disrupted pigment homeostasis under chemical stress [126].

Differential sensitivity between cyanobacteria and green algae to agrochemicals has been widely documented and is often attributed to structural and metabolic differences between prokaryotic and eukaryotic phototrophs. Cyanobacteria and green algae can respond differently to the same pesticides, with some cyanobacteria being less sensitive than green algae in acute toxicity tests. In comparisons involving organotins and pyrethroid pesticides, cyanobacteria were found to be substantially

less sensitive than green algae, suggesting that group-level differences can influence community responses to chemical stressors [113]. Similarly, in multi-species toxicity assays with carbamate insecticides, cyanobacteria often exhibited lower sensitivity than green algae, reflecting broader taxonomic patterns arising from distinct cellular and biochemical architectures [127]. From an ecological perspective, species-specific differences in pigment responses may influence competitive dynamics in contaminated aquatic systems. Light stress and chemical exposure frequently co-occur in shallow or eutrophic environments, and their interaction can amplify oxidative and physiological stress [3][128]. In *C. vulgaris*, Chl-b Chl-a^{-1} ratios increased under Ortiva and Basar, particularly from day 4 under SL, indicating selective downregulation of Chl b and adjustment of light-harvesting pigments to optimize PSII energy transfer. By day 7, ratios converged across treatments, suggesting partial acclimation and maintenance of pigment balance under prolonged stress.

Pesticide- and light-dependent modulation of PSII efficiency

In both species, F_v/F_m was strongly influenced by pesticide exposure, light intensity, and their interaction. Significant three-way interactions indicate that pesticide effects depend on both light conditions and exposure duration, highlighting the importance of considering multiple stressors simultaneously. F_v/F_m is widely used as an overall indicator for plant performance and typically declines under adverse living conditions. Under optimal conditions, healthy green microalgae exhibit F_v/F_m values between 0.65 and 0.75 [129], whereas sustained reductions reflect suboptimal living conditions, e.g., environmental stress. In the present experiment, we detected strong early reductions under Ortiva and Basar—particularly in *C. vulgaris*— which indicate direct or indirect interference with PSII function. A key finding was the contrasting response patterns between *C. vulgaris* (eukaryotic green alga) and *S. leopoliensis* (cyanobacterium). In *C. vulgaris*, Ortiva caused a pronounced initial decline in F_v/F_m, followed by substantial recovery over time, whereas Basar induced a stronger and more persistent suppression, especially under SL. In contrast, *S. leopoliensis* exhibited generally lower absolute F_v/F_m values and more variable, light-dependent responses, with Basar producing sustained inhibition and Ortiva inducing transient photoinhibitory effects. These interspecific differences are consistent with previous findings showing that cyanobacteria and green algae differ in photosystem organization, repair capacity, and susceptibility to xenobiotics [130]. Cyanobacteria possess distinct light-harvesting complexes (phycobilisomes) and may respond differently to oxidative or membrane-targeting stressors compared to chlorophytes, whose thylakoid membrane organization and PSII repair dynamics differ structurally and functionally [131]. The stronger suppression observed under Basar, particularly in *C. vulgaris*, suggests prolonged interference with PSII electron transport or enhanced oxidative stress, which impairs the D1 protein of PSII and delays repair cycles [132].

An important outcome of the GLMMs was the significant modulation of pesticide effects by light exposure. Under SL, both Ortiva and Basar showed lower F_v/F_m values than under WL, particularly during the early and intermediate exposure periods. This pattern suggests that SL immediately amplified pesticide-induced photoinhibition. When pesticide exposure simultaneously disrupts photochemical or membrane processes, the combined stress can exceed the capacity of photoprotective mechanisms, leading to greater reductions in F_v/F_m [122].

Both species exhibited temporal increases in F_v/F_m relative to day 0, indicating acclimation and/or partial recovery over time. The recovery observed under Ortiva exposure in *C. vulgaris* suggests reversible or transient inhibition rather than irreversible PSII damage. Under Basar, particularly in SL, F_v/F_m declined continuously, indicating damage. Sustained suppression of F_v/F_m over several days is often associated with chronic photoinhibition or structural membrane damage [133][134]. However, F_v/F_m alone may not fully reflect damage to the photosynthetic apparatus, as pigment analyses showed that chlorophyll content and pigment composition can change even when photochemical efficiency is partly maintained, indicating that pigment degradation and chloroplast dysfunction may not always be captured by F_v/F_m alone [135][136].

Pesticide exposure reduced photosynthetic efficiency in both microalgae and cyanobacteria, with effects strongly modulated by compound type and environmental conditions. As F_v/F_m is tightly linked to primary productivity, sustained reductions may impact carbon fixation and reshape community composition, with consequences for ecosystem functioning. PAM fluorometry revealed pronounced context dependency, with significant pesticide × light × time interactions indicating treatment-specific effects on growth and photosynthetic performance under combined stress.

Rapid Light Curves (RLC)

To further understand how structural PSII impairment translates into functional limitations, RLC parameters were analyzed. The patterns observed in α, ETR_{max}, and I_K closely mirrored the temporal dynamics of F_v/F_m, indicating that pesticide-induced changes in functional electron transport were directly linked to alterations in PSII efficiency. In *C. vulgaris*, Basar caused substantial reductions in F_v/F_m under strong irradiance, which paralleled marked declines in α and ETR_{max}. The simultaneous suppression of ETR_{max} and light-dependent electron transport suggests direct impairment at the PSII reaction centers rather than solely downstream metabolic limitation, consistent with Ralph and Gademann's [137] RLC interpretations. The increase in I_K under Basar indicates that SL was required to reach photosynthetic saturation, suggesting reduced light-use efficiency and altered energy allocation.

Ortiva exhibited a different pattern. Although F_v/F_m was strongly reduced during early exposure, it increased significantly over time, indicating recovery of PSII efficiency.

This recovery corresponded with relatively high ETR_{max} values, particularly under WL. The combination of transient F_v/F_m suppression and maintained electron transport capacity suggests reversible photoinhibitory effects rather than persistent structural damage [138]. The increased I_K observed under Ortiva further supports the hypothesis of modified light-response characteristics and photoprotective adjustment.

In *S. leopoliensis*, Basar induced sustained reductions in F_v/F_m, particularly under SL, accompanied by pronounced decreases in α, ETR_{max}, and I_K. The alignment between structural impairment (F_v/F_m) and functional suppression (RLC parameters) demonstrates that Basar exerted a persistent inhibitory effect on both the efficiency and capacity of the photosynthetic apparatus. The stronger suppression under elevated irradiance suggests an interaction between chemical stress and light-driven photoinhibition, a phenomenon frequently observed when xenobiotic exposure compromises PSII repair capacity under high light intensities [139]. In contrast to *C. vulgaris*, the reduction in I_K in *S. leopoliensis* indicates a diminished ability to sustain electron transport at increasing irradiance, reflecting lower physiological plasticity under combined stress.

The observed differential sensitivity of microalgae on *C. vulgaris* and *S. leopoliensis* to pesticide exposure may have important implications for aquatic ecosystem. As primary producers, microalgae underpin food web structure and biogeochemical cycling; thus, pesticide-induced alterations in biomass and pigment composition may cascade across trophic levels. The pronounced effects under elevated light suggest that environmental co-stressors can amplify toxicity in natural systems, particularly in shallow, high-irradiance waters. Such interactions may drive shifts in community composition toward more tolerant taxa, with potential consequences for ecosystem functioning and stability. These findings underscore the need to incorporate multiple, interacting environmental factors into pesticide risk assessment, as single-stressor laboratory studies may underestimate impacts under realistic field conditions.

5. Conclusion

Our results suggest that pesticide runoff has significant impacts on the phototrophic community; growth and photosynthetic response of the microalgae species *Chlorella vulgaris* and the cyanoprokaryote *Synechococcus leopoliensis* were strongly modulated by the type of pesticide, irradiance supply, and exposure duration. Testing across this broad concentration range allows evaluation of both sublethal and high-dose effects, providing insight into the tolerance thresholds of *C. vulgaris* and *S. leopoliensis* under pesticide stress. These results are critical for understanding potential ecological risks

associated with varying pesticide concentrations in aquatic systems and can inform safer agricultural application practices.

The integration of multiple growth parameters and physiological indicators revealed consistent interactions among pesticide, light, and time, highlighting that chemical stress does not act independently of environmental conditions. While OD generally serves as a convenient proxy for biomass, our results show that formulation-specific properties, such as Basar emulsions, can induce turbidity, decoupling OD from pigment-based biomass estimates, underscoring the need to combine OD with ChlF or other functional indicators for accurate assessments.

Among the tested pesticides, Basar caused the most persistent impairment of photosynthetic performance, including reductions in F_v/F_m, electron transport capacity, and pigment homeostasis, particularly under SL. Ortiva induced transient, partially reversible effects, whereas Teppeki had comparatively moderate impacts. Species-specific responses were also evident: *S. leopoliensis* displayed dynamic photoprotective modulation but was vulnerable to disrupted carotenoid balance under certain treatments, while *C. vulgaris* exhibited stronger suppression of PSII performance yet retained capacity for pigment reallocation and partial recovery over time. These differences underscore the importance of accounting for taxon-specific physiology in ecotoxicological evaluations. Overall, the findings highlight that pesticide impacts extend beyond simple growth inhibition to affect cellular resource allocation, photoacclimation capacity, and photosynthetic function. Accurate ecological risk assessment in freshwater systems, therefore, requires accounting for interacting environmental stressors, such as light intensity and exposure duration, as these factors can amplify or modify toxic effects, ultimately influencing primary productivity, community composition, and ecosystem resilience.

The vulnerability of microbial phototrophs to pesticide contamination highlights the need for stricter regulations on pesticide use and runoff management to prevent further ecological damage. Continued research into the specific mechanisms of toxicity and their long-term impacts is essential to develop mitigation strategies and protect aquatic environments from the detrimental effects of pesticide pollution.

Supplementary Files

Table 1: Recipe for the BG11 provided by the lab technician

Component	Stock Solution (g/100 mL)	Volume Added to Nutrient Solution (mL)
$NaNO_3$	15	10
$K_2HPO_4 \cdot 3H_2O$	0.4	10
$MgSO_4 \cdot 7H_2O$	0.75	10
$CaCl_2 \cdot 2H_2O$	0.36	10
Citric acid	0.06	10
Ferric ammonium citrate	0.06	10
EDTA (disodium salt)	0.01	10
Na_2CO_3	0.2	10
Micronutrient solution*	–	1
Deionized or distilled water	–	919

***composition of the micronutrient solution (from Kuhl and Lorenzen 1964)**

Add to 1000 ml of deionized or distilled water:

Component	Amount (mg)
H_3BO_3	61.0
$MnSO_4 \cdot H_2O$	169.0
$ZnSO_4 \cdot 7H_2O$	287.0
$CuSO_4 \cdot 5H_2O$	2.5
$(NH_4)_6Mo_7O_{24} \cdot 4H_2O$	12.5

Table 2. Sample lay out of 12-well costar plating. Six consecutive wells were intended for each of the concentrations.

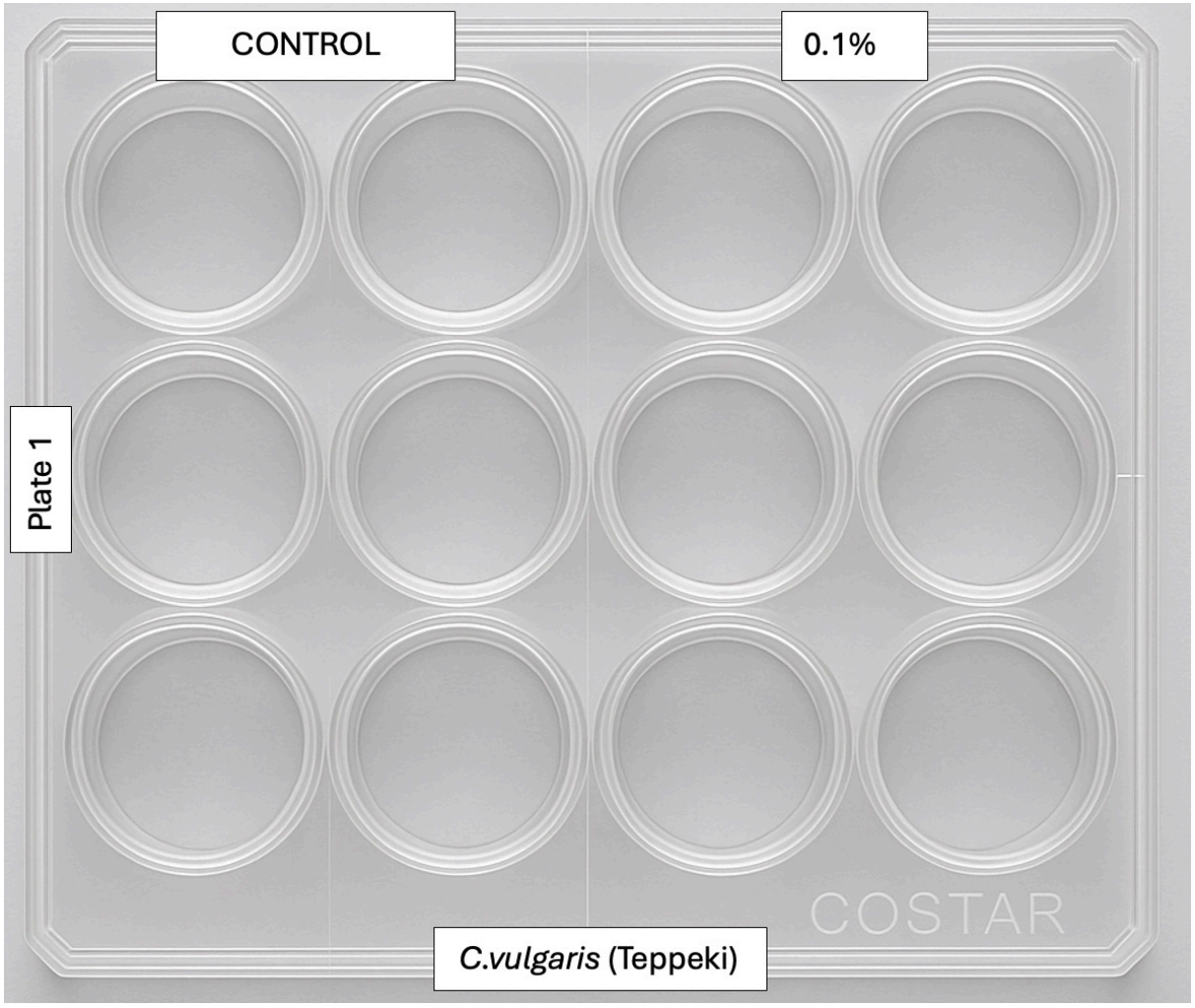


Table 3: Concentration in the dilution of pesticides using the costar plate

Final Concentration (% of Field Rate)	**Source (%)**	**Stock Pesticide (µL)**	**Medium + Culture (µL)**
25	1000	100	3900
10	1000	40	3960
5	200	100	3900
1	200	20	3980
0.1	10	40	3960
Control	–	0	4000

Table 4: Volumes of Pesticide Stock Solutions Added to the Bubble Column Reactors

Pesticide	**Final %**	**Stock Used (%)**	**Volume (mL) per 1L Column**	**Total Volume (mL) for 4 Columns (4L)**	**Medium + Culture (mL)**
Teppeki	5	200 stock	25	100	3900
Ortiva	5	200 stock	25	100	3900
Basar	1	1000 stock	1	4	3996

Table 5: GLMM results for ChlF-based biomass proxy in Chlorella vulgaris under three pesticide treatments

GLMM – Basar

Model Info

Info		
Model Type	Mixed Model	Linear Mixed model for continuous y
Model	lmer	`Basar Chla` ~ 1 + B_Treat_code + B_time + B_Treat_code:B_time + (1 | B_Code)
Distribution	Gaussian	Normal distribution of residuals
Direction	y	Dependend variable scores
Optimizer	bobyqa	
DF method	Satterthwaite	
Sample size	112	
Converged	yes	
Y transform	none	
C.I. method	Wald	

Model Results

Model Fit

Type	R^2	df	LRT X^2	p
Conditional	0.963	28	375.055	<.001
Marginal	0.946	27	364.586	<.001

Fixed Effects Omnibus Tests

	F	df	df (res)	p
B_Treat_code	74.6	3	12.0	<.001
B_time	168.5	6	72.0	<.001
B_Treat_code ✻ B_time	48.4	18	72.0	<.001

Random Components

Groups	Name	Variance	SD	ICC
B_Code	(Intercept)	391	19.8	0.309
Residual		874	29.6	

Note. Number of Obs: 112 , Number of groups: B_Code 16

Post Hoc Tests

Post Hoc comparison: B_Treat_code

B_Treat_code	vs	B_Treat_code	Difference	SE	t	df	$p_{bonferroni}$
Bas_B_S	-	Bas_B_W	-4.96	16.1	-0.309	12.0	1.000
Bas_B_S	-	Bas_C_S	-179.07	16.1	-11.153	12.0	<.001
Bas_B_S	-	Bas_C_W	-164.86	16.1	-10.268	12.0	<.001
Bas_B_W	-	Bas_C_S	-174.11	16.1	-10.844	12.0	<.001
Bas_B_W	-	Bas_C_W	-159.89	16.1	-9.959	12.0	<.001
Bas_C_S	-	Bas_C_W	14.21	16.1	0.885	12.0	1.000

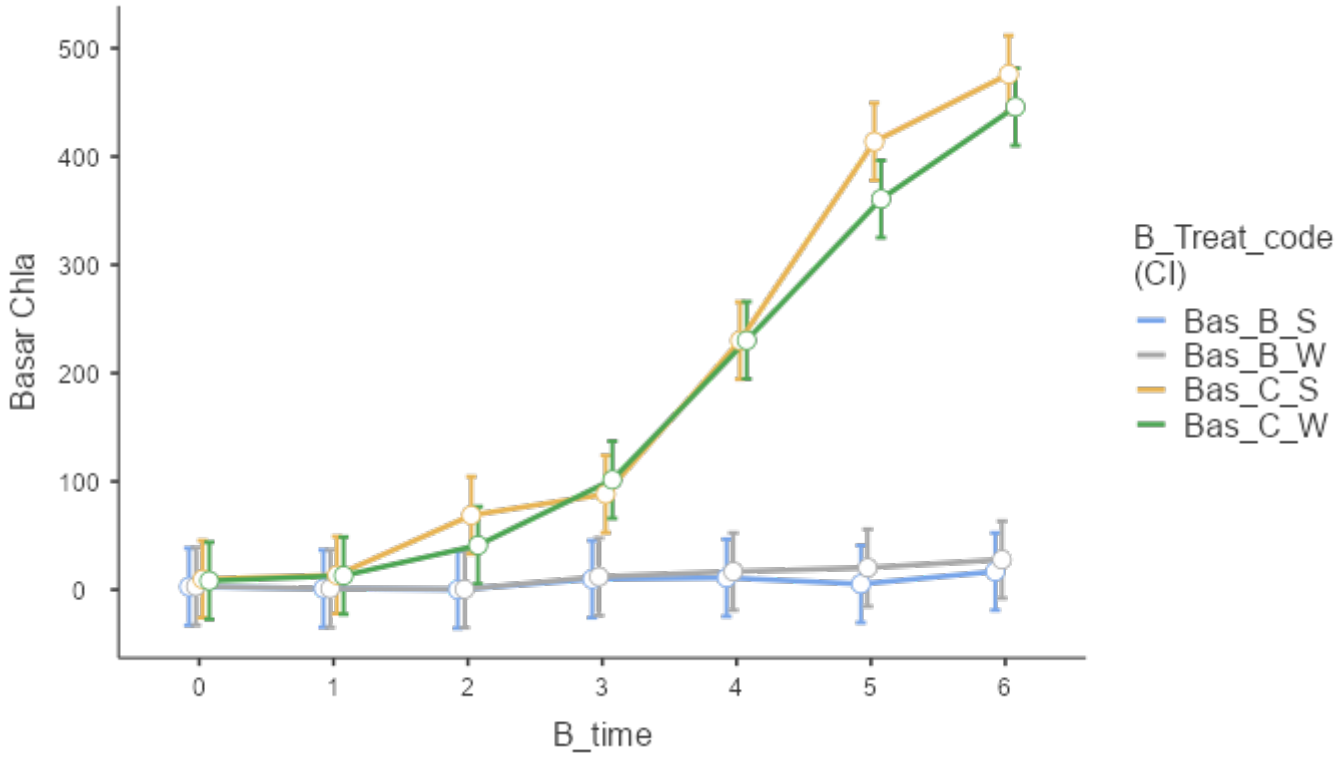
Estimated Marginal Means

Estimate Marginal Means - B_Treat_code ✻ B_time

					95% Confidence Intervals	
B_Treat_code	B_time	Mean	SE	df	Lower	Upper
Bas_B_S	0	2.750	17.8	53.4	-32.90	38.4
Bas_B_S	1	1.000	17.8	53.4	-34.65	36.7
Bas_B_S	2	6.44e-13	17.8	53.4	-35.65	35.7
Bas_B_S	3	9.750	17.8	53.4	-25.90	45.4
Bas_B_S	4	11.000	17.8	53.4	-24.65	46.7
Bas_B_S	5	5.250	17.8	53.4	-30.40	40.9

Bas_B_S	6	16.750	17.8	53.4	-18.90	52.4
Bas_B_W	0	3.000	17.8	53.4	-32.65	38.7
Bas_B_W	1	1.000	17.8	53.4	-34.65	36.7
Bas_B_W	2	0.500	17.8	53.4	-35.15	36.2
Bas_B_W	3	12.000	17.8	53.4	-23.65	47.7
Bas_B_W	4	16.750	17.8	53.4	-18.90	52.4
Bas_B_W	5	20.250	17.8	53.4	-15.40	55.9
Bas_B_W	6	27.750	17.8	53.4	-7.90	63.4
Bas_C_S	0	9.750	17.8	53.4	-25.90	45.4
Bas_C_S	1	13.500	17.8	53.4	-22.15	49.2
Bas_C_S	2	68.750	17.8	53.4	33.10	104.4
Bas_C_S	3	88.250	17.8	53.4	52.60	123.9
Bas_C_S	4	230.000	17.8	53.4	194.35	265.7
Bas_C_S	5	413.750	17.8	53.4	378.10	449.4
Bas_C_S	6	476.000	17.8	53.4	440.35	511.7
Bas_C_W	0	8.250	17.8	53.4	-27.40	43.9
Bas_C_W	1	13.000	17.8	53.4	-22.65	48.7
Bas_C_W	2	41.000	17.8	53.4	5.35	76.7
Bas_C_W	3	101.500	17.8	53.4	65.85	137.2
Bas_C_W	4	230.250	17.8	53.4	194.60	265.9
Bas_C_W	5	360.750	17.8	53.4	325.10	396.4
Bas_C_W	6	445.750	17.8	53.4	410.10	481.4

Results Plots



GLMM – Ortiva

Model Info

Info		
Model Type	Mixed Model	Linear Mixed model for continuous y
Model	lmer	`Ortiva Chla` ~ 1 + O_Treat_code + O_time + O_Treat_code:O_time + (1 | O_Code)
Distribution	Gaussian	Normal distribution of residuals
Direction	y	Dependend variable scores
Optimizer	bobyqa	
DF method	Satterthwaite	
Sample size	112	
Converged	yes	
Y transform	none	
C.I. method	Wald	

Model Results

Model Fit

Type	R^2	df	LRT X^2	p
Conditional	0.947	28	346.230	<.001
Marginal	0.939	27	345.973	<.001

Fixed Effects Omnibus Tests

	F	df	df (res)	p
O_Treat_code	53.0	3	12.0	<.001
O_time	230.1	6	72.0	<.001
O_Treat_code ✻ O_time	13.7	18	72.0	<.001

Random Components

Groups	Name	Variance	SD	ICC
O_Code	(Intercept)	199	14.1	0.127
Residual		1364	36.9	

Note. Number of Obs: 112 , Number of groups: O_Code 16

Post Hoc Tests

Post Hoc comparison: O_Treat_code

Comparison							
O_Treat_code	vs	O_Treat_code	Difference	SE	t	df	$p_{bonferroni}$
Ort_C_S	-	Ort_C_W	3.36	14.0	0.239	12.0	1.000
Ort_C_S	-	Ort_O_S	154.79	14.0	11.032	12.0	<.001
Ort_C_S	-	Ort_O_W	48.86	14.0	3.482	12.0	.027
Ort_C_W	-	Ort_O_S	151.43	14.0	10.793	12.0	<.001
Ort_C_W	-	Ort_O_W	45.50	14.0	3.243	12.0	.042
Ort_O_S	-	Ort_O_W	-105.93	14.0	-7.550	12.0	<.001

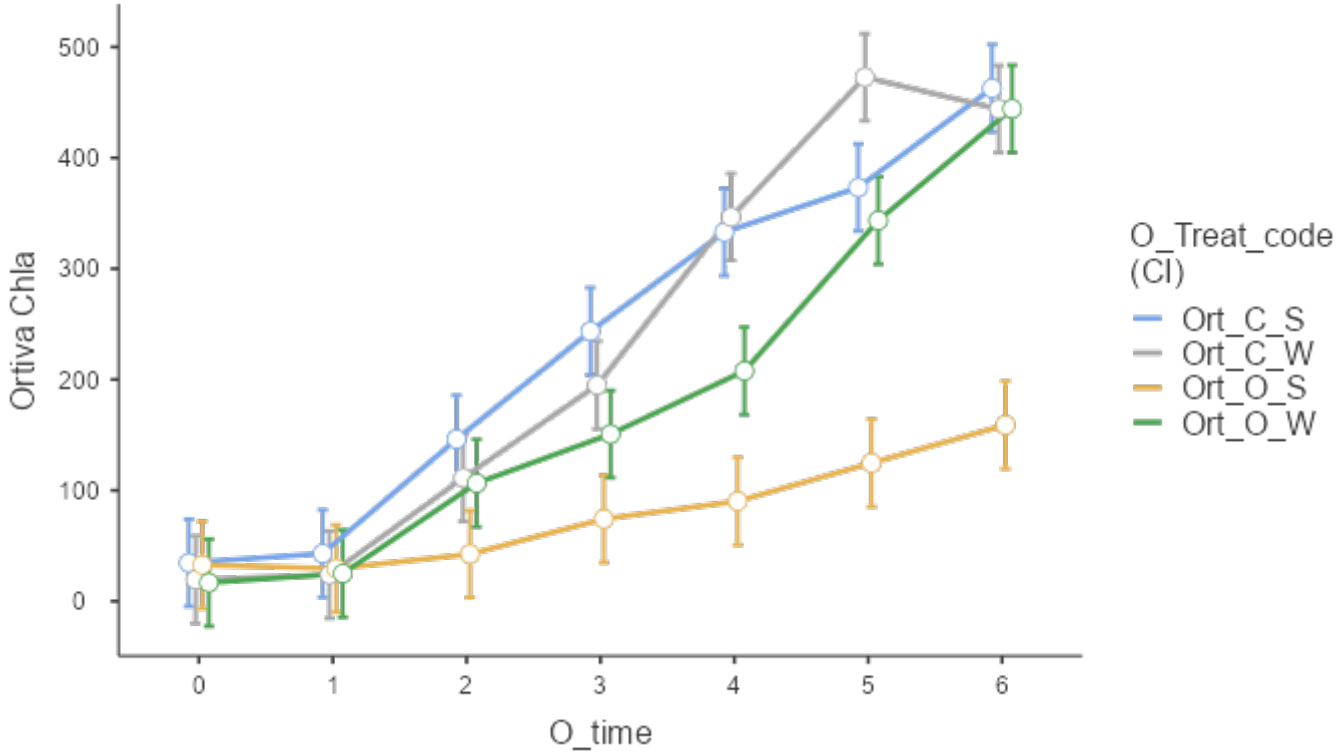
Estimated Marginal Means

Estimate Marginal Means - O_Treat_code * O_time

					95% Confidence Intervals	
O_Treat_code	O_time	Mean	SE	df	Lower	Upper
Ort_C_S	0	34.7	19.8	76.6	-4.61	74.1
Ort_C_S	1	43.0	19.8	76.6	3.64	82.4
Ort_C_S	2	146.2	19.8	76.6	106.89	185.6
Ort_C_S	3	243.5	19.8	76.6	204.14	282.9
Ort_C_S	4	333.0	19.8	76.6	293.64	372.4
Ort_C_S	5	373.2	19.8	76.6	333.89	412.6
Ort_C_S	6	462.7	19.8	76.6	423.39	502.1
Ort_C_W	0	19.5	19.8	76.6	-19.86	58.9
Ort_C_W	1	24.0	19.8	76.6	-15.36	63.4
Ort_C_W	2	111.3	19.8	76.6	71.89	150.6
Ort_C_W	3	195.0	19.8	76.6	155.64	234.4
Ort_C_W	4	346.5	19.8	76.6	307.14	385.9
Ort_C_W	5	472.8	19.8	76.6	433.39	512.1
Ort_C_W	6	444.0	19.8	76.6	404.64	483.4
Ort_O_S	0	32.8	19.8	76.6	-6.61	72.1
Ort_O_S	1	29.5	19.8	76.6	-9.86	68.9
Ort_O_S	2	42.5	19.8	76.6	3.14	81.9
Ort_O_S	3	74.3	19.8	76.6	34.89	113.6
Ort_O_S	4	90.3	19.8	76.6	50.89	129.6
Ort_O_S	5	124.8	19.8	76.6	85.39	164.1
Ort_O_S	6	159.0	19.8	76.6	119.64	198.4
Ort_O_W	0	16.8	19.8	76.6	-22.61	56.1
Ort_O_W	1	25.0	19.8	76.6	-14.36	64.4
Ort_O_W	2	106.5	19.8	76.6	67.14	145.9
Ort_O_W	3	150.8	19.8	76.6	111.39	190.1
Ort_O_W	4	207.8	19.8	76.6	168.39	247.1
Ort_O_W	5	343.5	19.8	76.6	304.14	382.9
Ort_O_W	6	444.3	19.8	76.6	404.89	483.6

Results Plots

O_time ✻ O_Treat_code



GLMM – Teppeki

Model Info

Info		
Model Type	Mixed Model	Linear Mixed model for continuous y
Model	lmer	`Teppeki Chla` ~ 1 + T_Treat_code + T_time + T_Treat_code:T_time + (1 | T_Code)
Distribution	Gaussian	Normal distribution of residuals
Direction	y	Dependend variable scores
Optimizer	bobyqa	
DF method	Satterthwaite	
Sample size	96	
Converged	yes	
Y transform	none	
C.I. method	Wald	

Model Results

Model Fit

Type	R^2	df	LRT X^2	p
Conditional	0.979	24	396.752	<.001
Marginal	0.979	23	396.752	<.001

Fixed Effects Omnibus Tests

	F	df	df (res)	p
T_Treat_code	0.541	3	72.0	.656
T_time	860.803	5	72.0	<.001
T_Treat_code ✻ T_time	7.458	15	72.0	<.001

Random Components

Groups	Name	Variance	SD	ICC
T_Code	(Intercept)	0	0.0	0.00
Residual		650	25.5	

Note. Number of Obs: 96 , Number of groups: T_Code 16

Post Hoc Tests

Post Hoc comparison: T_Treat_code

Comparison							
T_Treat_code	vs	T_Treat_code	Difference	SE	t	df	$p_{bonferroni}$
Tep_C_S	-	Tep_C_W	-4.792	7.36	-0.6509	72.0	1.000
Tep_C_S	-	Tep_T_S	-9.375	7.36	-1.2735	72.0	1.000
Tep_C_S	-	Tep_T_W	-4.542	7.36	-0.6170	72.0	1.000
Tep_C_W	-	Tep_T_S	-4.583	7.36	-0.6226	72.0	1.000
Tep_C_W	-	Tep_T_W	0.250	7.36	0.0340	72.0	1.000
Tep_T_S	-	Tep_T_W	4.833	7.36	0.6566	72.0	1.000

Estimated Marginal Means

Estimate Marginal Means - T_Treat_code * T_time

					95% Confidence Intervals	
T_Treat_code	T_time	Mean	SE	df	Lower	Upper
Tep_C_S	0	12.0	12.8	72.0	-13.4174	37.4
Tep_C_S	1	24.8	12.8	72.0	-0.6674	50.2
Tep_C_S	2	146.2	12.8	72.0	120.8326	171.7
Tep_C_S	3	307.5	12.8	72.0	282.0826	332.9
Tep_C_S	4	464.3	12.8	72.0	438.8326	489.7
Tep_C_S	5	315.5	12.8	72.0	290.0826	340.9
Tep_C_W	0	15.8	12.8	72.0	-9.6674	41.2
Tep_C_W	1	31.3	12.8	72.0	5.8326	56.7

Estimate Marginal Means - T_Treat_code * T_time

Tep_C_W	2	111.3	12.8	72.0	85.8326	136.7
Tep_C_W	3	271.8	12.8	72.0	246.3326	297.2
Tep_C_W	4	432.3	12.8	72.0	406.8326	457.7
Tep_C_W	5	436.8	12.8	72.0	411.3326	462.2
Tep_T_S	0	12.8	12.8	72.0	-12.6674	38.2
Tep_T_S	1	25.5	12.8	72.0	0.0826	50.9
Tep_T_S	2	142.5	12.8	72.0	117.0826	167.9
Tep_T_S	3	317.0	12.8	72.0	291.5826	342.4
Tep_T_S	4	473.8	12.8	72.0	448.3326	499.2
Tep_T_S	5	355.0	12.8	72.0	329.5826	380.4
Tep_T_W	0	15.8	12.8	72.0	-9.6674	41.2
Tep_T_W	1	29.0	12.8	72.0	3.5826	54.4
Tep_T_W	2	106.5	12.8	72.0	81.0826	131.9
Tep_T_W	3	260.3	12.8	72.0	234.8326	285.7
Tep_T_W	4	429.5	12.8	72.0	404.0826	454.9
Tep_T_W	5	456.5	12.8	72.0	431.0826	481.9

Results Plots

T_time ✻ T_Treat_code

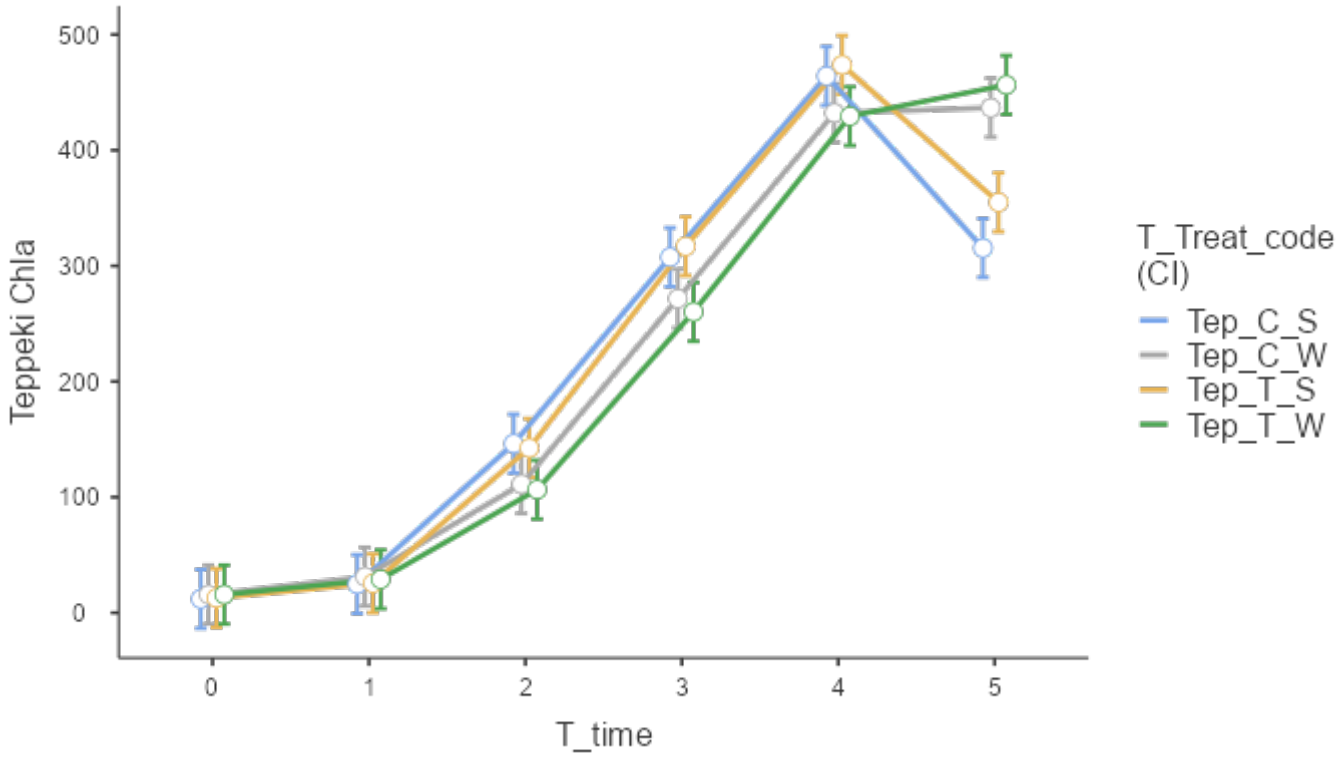


Table 6: GLMM result for ChlF-based biomass proxy in Synechococcus leopoliensis under three pesticide treatments

GLMM – Basar

Model Info

Info		
Model Type	Mixed Model	Linear Mixed model for continuous y
Model	lmer	`Basar Chla` ~ 1 + B_Treat_code + B_time + B_Treat_code:B_time + (1 | B_Code)
Distribution	Gaussian	Normal distribution of residuals
Direction	y	Dependend variable scores
Optimizer	bobyqa	
DF method	Satterthwaite	
Sample size	128	
Converged	yes	
Y transform	none	
C.I. method	Wald	

Model Results

Model Fit

Type	R^2	df	LRT X^2	p
Conditional	0.975	32	484.423	<.001
Marginal	0.965	31	484.428	<.001

Fixed Effects Omnibus Tests

	F	df	df (res)	p
B_Treat_code	49.7	3	12.0	<.001
B_time	522.0	7	84.0	<.001
B_Treat_code ✻ B_time	30.6	21	84.0	<.001

Random Components

Groups	Name	Variance	SD	ICC
B_Code	(Intercept)	32.6	5.71	0.280
Residual		83.7	9.15	

Note. Number of Obs: 128 , Number of groups: B_Code 16

Post Hoc Tests

Post Hoc comparison: B_Treat_code

Comparison							
B_Treat_code	vs	B_Treat_code	Difference	SE	t	df	$p_{bonferroni}$
Bas_B_S	-	Bas_B_W	-12.5	4.64	-2.69	12.0	.117
Bas_B_S	-	Bas_C_S	-52.2	4.64	-11.25	12.0	<.001
Bas_B_S	-	Bas_C_W	-34.2	4.64	-7.38	12.0	<.001
Bas_B_W	-	Bas_C_S	-39.7	4.64	-8.55	12.0	<.001
Bas_B_W	-	Bas_C_W	-21.7	4.64	-4.68	12.0	.003
Bas_C_S	-	Bas_C_W	18.0	4.64	3.87	12.0	.013

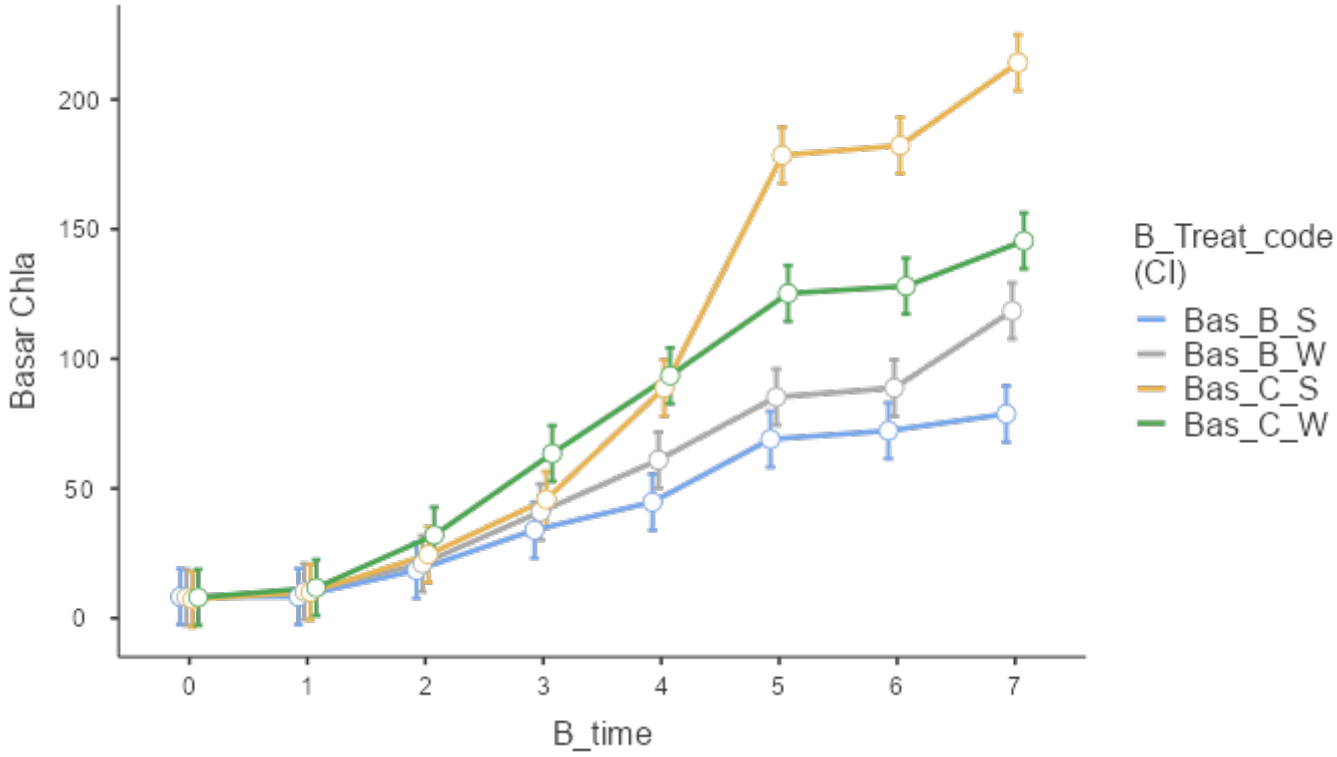
Estimated Marginal Means

Estimate Marginal Means - B_Treat_code * B_time

					95% Confidence Intervals	
B_Treat_code	B_time	Mean	SE	df	Lower	Upper
Bas_B_S	0	8.25	5.39	61.9	-2.527	19.0
Bas_B_S	1	8.25	5.39	61.9	-2.527	19.0
Bas_B_S	2	18.50	5.39	61.9	7.723	29.3
Bas_B_S	3	34.00	5.39	61.9	23.223	44.8
Bas_B_S	4	44.75	5.39	61.9	33.973	55.5
Bas_B_S	5	69.00	5.39	61.9	58.223	79.8
Bas_B_S	6	72.25	5.39	61.9	61.473	83.0
Bas_B_S	7	78.75	5.39	61.9	67.973	89.5
Bas_B_W	0	8.00	5.39	61.9	-2.777	18.8
Bas_B_W	1	10.25	5.39	61.9	-0.527	21.0
Bas_B_W	2	21.00	5.39	61.9	10.223	31.8
Bas_B_W	3	41.00	5.39	61.9	30.223	51.8
Bas_B_W	4	61.00	5.39	61.9	50.223	71.8
Bas_B_W	5	85.25	5.39	61.9	74.473	96.0
Bas_B_W	6	88.75	5.39	61.9	77.973	99.5
Bas_B_W	7	118.50	5.39	61.9	107.723	129.3
Bas_C_S	0	7.25	5.39	61.9	-3.527	18.0
Bas_C_S	1	10.00	5.39	61.9	-0.777	20.8
Bas_C_S	2	24.50	5.39	61.9	13.723	35.3
Bas_C_S	3	45.75	5.39	61.9	34.973	56.5
Bas_C_S	4	88.75	5.39	61.9	77.973	99.5
Bas_C_S	5	178.50	5.39	61.9	167.723	189.3
Bas_C_S	6	182.25	5.39	61.9	171.473	193.0
Bas_C_S	7	214.25	5.39	61.9	203.473	225.0
Bas_C_W	0	8.00	5.39	61.9	-2.777	18.8
Bas_C_W	1	11.75	5.39	61.9	0.973	22.5
Bas_C_W	2	32.00	5.39	61.9	21.223	42.8
Bas_C_W	3	63.50	5.39	61.9	52.723	74.3
Bas_C_W	4	93.50	5.39	61.9	82.723	104.3

Bas_C_W	5	125.25	5.39	61.9	114.473	136.0
Bas_C_W	6	128.00	5.39	61.9	117.223	138.8
Bas_C_W	7	145.50	5.39	61.9	134.723	156.3

Results Plots



GLMM – Ortiva

Model Info

Info		
Model Type	Mixed Model	Linear Mixed model for continuous y
Model	lmer	`Ortiva Chla` ~ 1 + O_Treat_code + O_time + O_Treat_code:O_time + (1 | O_Code)
Distribution	Gaussian	Normal distribution of residuals
Direction	y	Dependend variable scores
Optimizer	bobyqa	
DF method	Satterthwaite	
Sample size	128	
Converged	yes	
Y transform	none	

C.I. method Wald

Model Results

Model Fit

Type	R^2	df	LRT X^2	p
Conditional	0.938	32	374.366	<.001
Marginal	0.925	31	370.916	<.001

Fixed Effects Omnibus Tests

	F	df	df (res)	p
O_Treat_code	49.4	3	12.0	<.001
O_time	109.5	7	84.0	<.001
O_Treat_code ✻ O_time	35.3	21	84.0	<.001

Random Components

Groups	Name	Variance	SD	ICC
O_Code	(Intercept)	26.8	5.18	0.177
Residual		124.6	11.16	

Note. Number of Obs: 128 , Number of groups: O_Code 16

Post Hoc Tests

Post Hoc comparison: O_Treat_code

Comparison							
O_Treat_code	vs	O_Treat_code	Difference	SE	t	df	$p_{bonferroni}$
Ort_C_S	-	Ort_C_W	22.8	4.61	4.95	12.0	.002
Ort_C_S	-	Ort_O_S	47.6	4.61	10.33	12.0	<.001
Ort_C_S	-	Ort_O_W	47.6	4.61	10.33	12.0	<.001
Ort_C_W	-	Ort_O_S	24.8	4.61	5.39	12.0	<.001
Ort_C_W	-	Ort_O_W	24.8	4.61	5.39	12.0	<.001
Ort_O_S	-	Ort_O_W	-7.11e−15	4.61	-1.54e−15	12.0	1.000

Estimated Marginal Means

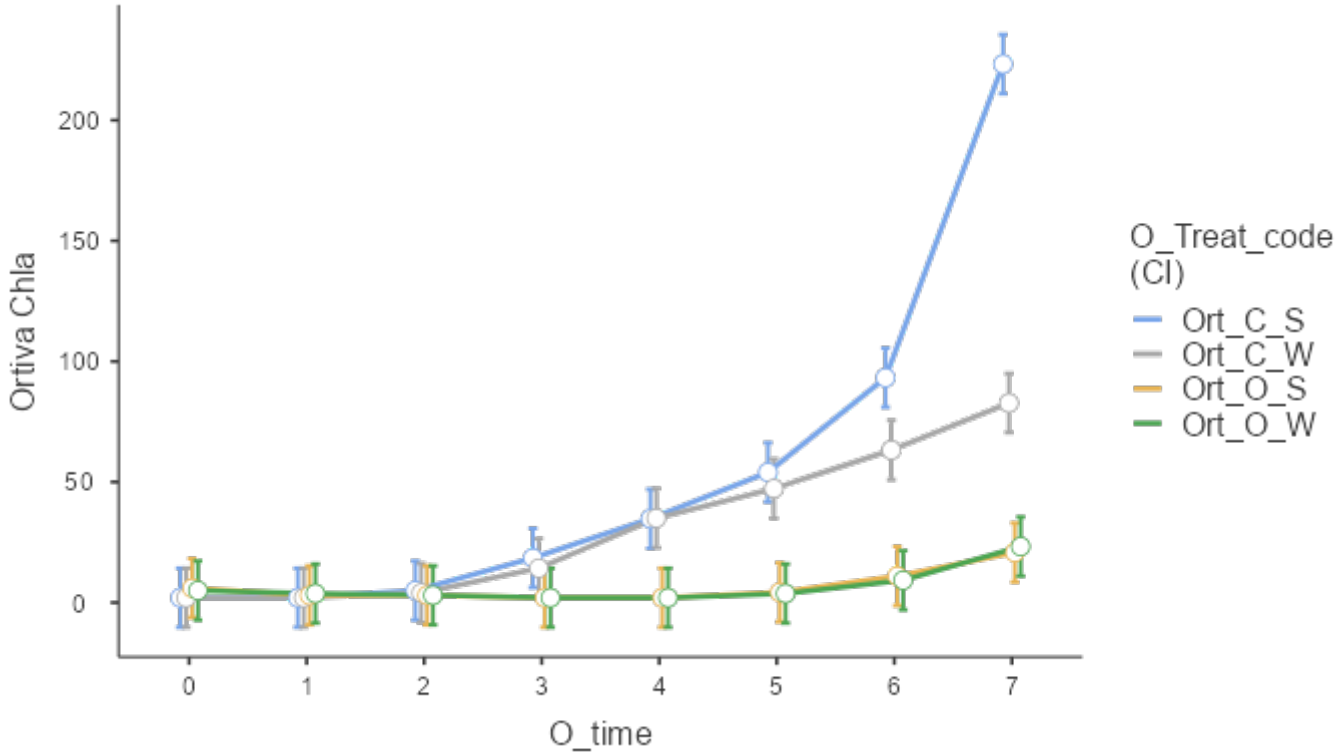
Estimate Marginal Means - O_Treat_code * O_time

					95% Confidence Intervals	
O_Treat_code	O_time	Mean	SE	df	Lower	Upper
Ort_C_S	0	2.00	6.15	78.7	-10.25	14.2
Ort_C_S	1	2.00	6.15	78.7	-10.25	14.2
Ort_C_S	2	5.00	6.15	78.7	-7.25	17.2
Ort_C_S	3	18.50	6.15	78.7	6.25	30.7
Ort_C_S	4	34.75	6.15	78.7	22.50	47.0
Ort_C_S	5	54.00	6.15	78.7	41.75	66.2
Ort_C_S	6	93.25	6.15	78.7	81.00	105.5
Ort_C_S	7	223.25	6.15	78.7	211.00	235.5
Ort_C_W	0	2.00	6.15	78.7	-10.25	14.2
Ort_C_W	1	2.00	6.15	78.7	-10.25	14.2
Ort_C_W	2	4.00	6.15	78.7	-8.25	16.2
Ort_C_W	3	14.25	6.15	78.7	2.00	26.5
Ort_C_W	4	35.00	6.15	78.7	22.75	47.2
Ort_C_W	5	47.25	6.15	78.7	35.00	59.5
Ort_C_W	6	63.25	6.15	78.7	51.00	75.5
Ort_C_W	7	82.75	6.15	78.7	70.50	95.0
Ort_O_S	0	6.00	6.15	78.7	-6.25	18.2
Ort_O_S	1	3.00	6.15	78.7	-9.25	15.2
Ort_O_S	2	3.00	6.15	78.7	-9.25	15.2
Ort_O_S	3	2.00	6.15	78.7	-10.25	14.2
Ort_O_S	4	2.00	6.15	78.7	-10.25	14.2
Ort_O_S	5	4.25	6.15	78.7	-8.00	16.5
Ort_O_S	6	11.00	6.15	78.7	-1.25	23.2
Ort_O_S	7	20.75	6.15	78.7	8.50	33.0
Ort_O_W	0	5.00	6.15	78.7	-7.25	17.2
Ort_O_W	1	3.75	6.15	78.7	-8.50	16.0
Ort_O_W	2	3.00	6.15	78.7	-9.25	15.2
Ort_O_W	3	2.00	6.15	78.7	-10.25	14.2
Ort_O_W	4	2.00	6.15	78.7	-10.25	14.2

Ort_O_W	5	3.75	6.15	78.7	-8.50	16.0
Ort_O_W	6	9.25	6.15	78.7	-3.00	21.5
Ort_O_W	7	23.25	6.15	78.7	11.00	35.5

Results Plots

O_time ✻ O_Treat_code



GLMM - Teppeki

Model Info

Info		
Model Type	Mixed Model	Linear Mixed model for continuous y
Model	lmer	`Teppeki Chla` ~ 1 + T_Treat_code + T_time + T_Treat_code:T_time + (1 | T_Code)
Distribution	Gaussian	Normal distribution of residuals
Direction	y	Dependend variable scores
Optimizer	bobyqa	
DF method	Satterthwaite	
Sample size	128	
Converged	yes	

Y transform	none
C.I. method	Wald

Model Results

Model Fit

Type	R^2	df	LRT X^2	p
Conditional	0.956	32	429.700	<.001
Marginal	0.954	31	429.700	<.001

Fixed Effects Omnibus Tests

	F	df	df (res)	p
T_Treat_code	26.71	3	12.0	<.001
T_time	364.27	7	84.0	<.001
T_Treat_code ✻ T_time	3.91	21	84.0	<.001

Random Components

Groups	Name	Variance	SD	ICC
T_Code	(Intercept)	1.92	1.38	0.0289
Residual		64.46	8.03	

Note. Number of Obs: 128 , Number of groups: T_Code 16

Post Hoc Tests

Post Hoc comparison: T_Treat_code

Comparison							
T_Treat_code	vs	T_Treat_code	Difference	SE	t	df	$p_{bonferroni}$
Tep_C_S	-	Tep_C_W	-8.34	2.23	-3.736	12.0	.017
Tep_C_S	-	Tep_T_S	7.44	2.23	3.330	12.0	.036
Tep_C_S	-	Tep_T_W	-10.31	2.23	-4.618	12.0	.004
Tep_C_W	-	Tep_T_S	15.78	2.23	7.067	12.0	<.001
Tep_C_W	-	Tep_T_W	-1.97	2.23	-0.882	12.0	1.000
Tep_T_S	-	Tep_T_W	-17.75	2.23	-7.948	12.0	<.001

Estimated Marginal Means

Estimate Marginal Means - T_Treat_code ✻ T_time

					95% Confidence Intervals	
T_Treat_code	T_time	Mean	SE	df	Lower	Upper
Tep_C_S	0	11.00	4.07	95.4	2.913	19.1
Tep_C_S	1	8.75	4.07	95.4	0.663	16.8
Tep_C_S	2	16.25	4.07	95.4	8.163	24.3
Tep_C_S	3	43.75	4.07	95.4	35.663	51.8
Tep_C_S	4	47.75	4.07	95.4	39.663	55.8
Tep_C_S	5	82.50	4.07	95.4	74.413	90.6
Tep_C_S	6	94.75	4.07	95.4	86.663	102.8
Tep_C_S	7	96.50	4.07	95.4	88.413	104.6
Tep_C_W	0	15.75	4.07	95.4	7.663	23.8
Tep_C_W	1	10.50	4.07	95.4	2.413	18.6
Tep_C_W	2	20.50	4.07	95.4	12.413	28.6
Tep_C_W	3	52.00	4.07	95.4	43.913	60.1
Tep_C_W	4	62.25	4.07	95.4	54.163	70.3
Tep_C_W	5	85.00	4.07	95.4	76.913	93.1
Tep_C_W	6	105.50	4.07	95.4	97.413	113.6
Tep_C_W	7	116.50	4.07	95.4	108.413	124.6
Tep_T_S	0	15.25	4.07	95.4	7.163	23.3
Tep_T_S	1	8.75	4.07	95.4	0.663	16.8
Tep_T_S	2	16.50	4.07	95.4	8.413	24.6
Tep_T_S	3	37.50	4.07	95.4	29.413	45.6
Tep_T_S	4	42.75	4.07	95.4	34.663	50.8
Tep_T_S	5	55.00	4.07	95.4	46.913	63.1
Tep_T_S	6	71.75	4.07	95.4	63.663	79.8
Tep_T_S	7	94.25	4.07	95.4	86.163	102.3
Tep_T_W	0	10.75	4.07	95.4	2.663	18.8
Tep_T_W	1	10.75	4.07	95.4	2.663	18.8
Tep_T_W	2	20.50	4.07	95.4	12.413	28.6
Tep_T_W	3	54.50	4.07	95.4	46.413	62.6
Tep_T_W	4	58.00	4.07	95.4	49.913	66.1
Tep_T_W	5	88.50	4.07	95.4	80.413	96.6
Tep_T_W	6	114.75	4.07	95.4	106.663	122.8

Tep_T_W	7	126.00	4.07	95.4	117.913	134.1

Results Plots

T_time ✻ T_Treat_code

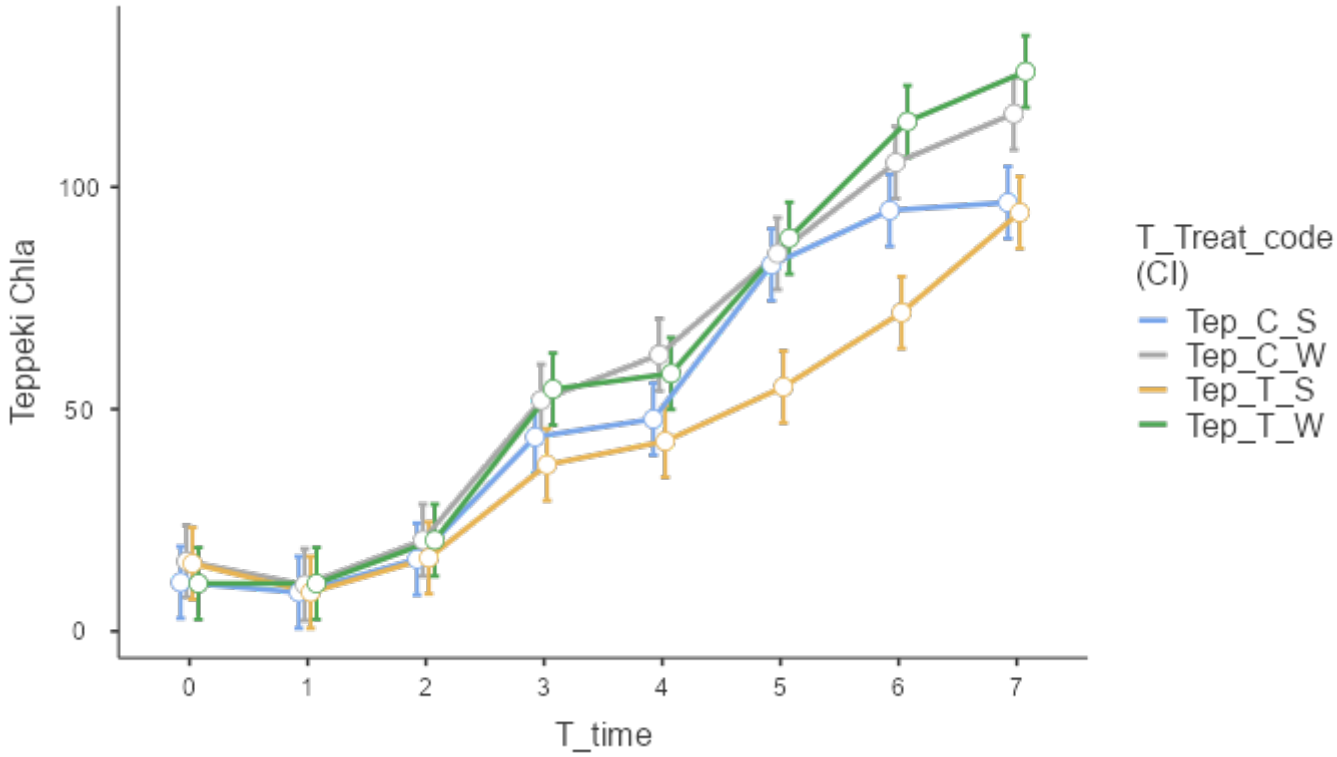


Table 7: GLMM results for Chla DM^{-1} in Chlorella vulgaris under three pesticide treatments

Component	Term	Value
Model type	Linear mixed model (REML)	lmer
Formula	Response	per_mil_chla_DM [‰]
Fixed effects	Structure	Treatment × Light × Day
Random effects	Structure	(1
Optimizer	Method	bobyqa
Test method	df approximation	Satterthwaite
Sample size	Observations	144
Groups	Replicates	16
Convergence	Status	Yes
REML criterion	Fit index	772.4

Random Effects

Group	Effect	Variance	Std. Dev.
Replicates	Intercept	0.00	0.00
Residual	—	26.21	5.12

Residual Diagnostics

Statistic	Value
Min	-2.8613
1Q	-0.3883
Median	-0.0185
3Q	0.2163
Max	3.7306

Fixed Effects (Linear Mixed Model)

Response: per mil Chl-a DM (‰)

Model: Treatment × Light × Day + (1 | Replicates)

Main Effects

Term	Estimate	SE	df	t	p-value	Signif.
(Intercept)	5.39092	1.47794	120	3.648	0.000393	***
Treatment Teppeki	0.11326	2.95589	120	0.038	0.969500	ns
Treatment Ortiva	-1.22390	2.95589	120	-0.414	0.679572	ns
Treatment Basar	1.03064	2.95589	120	0.349	0.727946	ns
Light Weak	-0.03831	2.09013	120	-0.018	0.985408	ns

Time Effect

Term	Estimate	SE	df	t	p-value	Signif.
Day 4	12.68772	2.09013	120	6.070	1.54e-08	***
Day 7	11.42825	2.09013	120	5.468	2.52e-07	***

Treatment × Light Interactions

Term	Estimate	SE	df	t	p-value	Signif.
Teppeki × Weak	0.03831	4.18026	120	0.009	0.992704	ns
Ortiva × Weak	0.15262	4.18026	120	0.037	0.970937	ns
Basar × Weak	0.03831	4.18026	120	0.009	0.992704	ns

Treatment × Day Interactions

Term	Estimate	SE	df	t	p-value	Signif.
Teppeki × Day 4	0.92087	4.18026	120	0.220	0.826020	ns
Ortiva × Day 4	-8.42719	4.18026	120	-2.016	0.046039	*
Basar × Day 4	-18.70444	4.18026	120	-4.474	1.75e-05	***

Term	Estimate	SE	df	t	p-value	Signif.
Teppeki × Day 7	9.23719	4.18026	120	2.210	0.029022	*
Ortiva × Day 7	-7.16376	4.18026	120	-1.714	0.089164	.
Basar × Day 7	-17.50664	4.18026	120	-4.188	5.40e-05	***

Light × Day Interactions

Term	Estimate	SE	df	t	p-value	Signif.
Weak × Day 4	3.01137	2.95589	120	1.019	0.310361	ns
Weak × Day 7	10.34122	2.95589	120	3.499	0.000657	***

Three-way Interactions

Term	Estimate	SE	df	t	p-value	Signif.
Teppeki × Weak × Day 4	1.60737	5.91177	120	0.272	0.786171	ns
Ortiva × Weak × Day 4	-2.42745	5.91177	120	-0.411	0.682088	ns
Basar × Weak × Day 4	-2.21734	5.91177	120	-0.375	0.708269	ns
Teppeki × Weak × Day 7	-2.78084	5.91177	120	-0.470	0.638930	ns
Ortiva × Weak × Day 7	-7.19662	5.91177	120	-1.217	0.225865	ns
Basar × Weak × Day 7	-9.84255	5.91177	120	-1.665	0.098540	.

Effect	Sum Sq	Mean Sq	NumDF	DenDF	F value	p-value	Signif.
Treatment	4609.4	1536.47	3	120	58.6176	< 2.2e-16	***
Light	189.5	189.49	1	120	7.2293	0.008193	**
Day	2138.4	1069.22	2	120	40.7915	3.045e-14	***
Treatment × Light	97.3	32.42	3	120	1.2370	0.299390	ns
Treatment × Day	2608.8	434.81	6	120	16.5882	7.381e-14	***
Light × Day	140.5	70.25	2	120	2.6801	0.072661	.
Treatment × Light × Day	104.0	17.33	6	120	0.6612	0.681052	ns

Table 8: GLMM results for Chla DM^{-1} in S. leopoliensis under three pesticide treatments

Linear mixed model summary (REML)

Component	Value
Model type	Linear mixed model (lmer)
Formula	per_mil_chla_DM[‰] ~ Treatment × Light × Day + (1

Component	Value
Data	df_clean
Optimizer	bobyqa
DF method	Satterthwaite
Sample size	144
Groups (Replicates)	16
Convergence	Yes
REML criterion	772.4

Random effects

Group	Effect	Variance	SD
Replicates	Intercept	0.00	0.00
Residual	—	26.21	5.12

Fixed effects

Predictor	Estimate	SE	df	t	p-value
(Intercept)	5.3909	1.4779	120	3.648	<0.001 ***
Treatment Teppeki	0.1133	2.9559	120	0.038	0.970
Treatment Ortiva	-1.2239	2.9559	120	-0.414	0.680
Treatment Basar	1.0306	2.9559	120	0.349	0.728
Light Weak	-0.0383	2.0901	120	-0.018	0.985
Day Day 4	12.6877	2.0901	120	6.070	<0.001 ***
Day Day 7	11.4283	2.0901	120	5.468	<0.001 ***
Teppeki × Light	0.0383	4.1803	120	0.009	0.993
Ortiva × Light	0.1526	4.1803	120	0.037	0.971
Basar × Light	0.0383	4.1803	120	0.009	0.993
Teppeki × Day 4	0.9209	4.1803	120	0.220	0.826
Ortiva × Day 4	-8.4272	4.1803	120	-2.016	0.046 *
Basar × Day 4	-18.7044	4.1803	120	-4.474	<0.001 ***
Teppeki × Day 7	9.2372	4.1803	120	2.210	0.029 *
Ortiva × Day 7	-7.1638	4.1803	120	-1.714	0.089
Basar × Day 7	-17.5066	4.1803	120	-4.188	<0.001 ***

Factor	Sum Sq	Mean Sq	df	F	p-value
Treatment	4609.4	1536.47	3	58.62	<0.001 ***
Light	189.5	189.49	1	7.23	0.008 **

Factor	Sum Sq	Mean Sq	df	F	p-value
Day	2138.4	1069.22	2	40.79	<0.001 ***
Treatment × Light	97.3	32.42	3	1.24	0.299
Treatment × Day	2608.8	434.81	6	16.59	<0.001 ***
Light × Day	140.5	70.25	2	2.68	0.073
Treatment × Light × Day	104.0	17.33	6	0.66	0.681

Table 9: GLMM results for Car Chl-a^{-1} *in Chlorella vulgaris under three pesticide treatments*

Component	Value
Model type	Linear mixed model (lmer)
Formula	Carotenoids/Chla ~ Treatment × Light × Day + (1
Data	df_clean
Optimization	bobyqa
REML criterion	-430
Observations	144
Groups (Replicates)	16
Convergence	Yes

Random effects

Group	Effect	Variance	Std. Dev.
Replicates	Intercept	3.313e-21	5.756e-11
Residual	—	1.167e-03	3.417e-02

Fixed effects (Carotenoids / Chla)

Predictor	Estimate	SE	df	t	p-value
(Intercept)	0.13330	0.00986	120	13.512	<0.001 ***
Treatment Teppeki	0.01525	0.01973	120	0.773	0.441
Treatment Ortiva	0.01423	0.01973	120	0.721	0.472
Treatment Basar	-0.00017	0.01973	120	-0.009	0.993
Light Weak	0.00500	0.01395	120	0.358	0.721
Day 4	-0.02833	0.01395	120	-2.031	0.045 *
Day 7	-0.01393	0.01395	120	-0.999	0.320
Teppeki × Light	-0.00500	0.02790	120	-0.179	0.858
Ortiva × Light	-0.00500	0.02790	120	-0.179	0.858
Basar × Light	-0.01633	0.02790	120	-0.585	0.559
Teppeki × Day 4	-0.01947	0.02790	120	-0.698	0.487

Predictor	Estimate	SE	df	t	p-value
Ortiva × Day 4	0.02464	0.02790	120	0.883	0.379
Basar × Day 4	0.21220	0.02790	120	7.607	<0.001 ***
Teppeki × Day 7	-0.02851	0.02790	120	-1.022	0.309
Ortiva × Day 7	-0.00219	0.02790	120	-0.078	0.938
Basar × Day 7	0.12130	0.02790	120	4.347	<0.001 ***
Light × Day 4	0.00007	0.01973	120	0.004	0.997
Light × Day 7	0.00361	0.01973	120	0.183	0.855
Teppeki × Light × Day 4	0.02310	0.03945	120	0.585	0.559
Ortiva × Light × Day 4	-0.01388	0.03945	120	-0.352	0.726
Basar × Light × Day 4	-0.08944	0.03945	120	-2.267	0.025 *
Teppeki × Light × Day 7	-0.01046	0.03945	120	-0.265	0.791
Ortiva × Light × Day 7	-0.03356	0.03945	120	-0.851	0.397
Basar × Light × Day 7	-0.04675	0.03945	120	-1.185	0.238

Type III analysis of fixed effects

Factor	Sum Sq	Mean Sq	df (Num)	df (Den)	F value	p-value
Treatment	0.1246	0.0415	3	120	35.565	<0.001 ***
Light	0.0061	0.0061	1	120	5.263	0.024 *
Day	0.0069	0.0035	2	120	2.969	0.055 .
Treatment × Light	0.0185	0.0062	3	120	5.289	0.002 **
Treatment × Day	0.1010	0.0168	6	120	14.421	<0.001 ***
Light × Day	0.0024	0.0012	2	120	1.048	0.354
Treatment × Light × Day	0.0087	0.0014	6	120	1.239	0.291

Table 10: GLMM results for Car Chl-a^{-1} *in S. leopoliensis under three pesticide treatments*

Model summary (Carotenoids / Chla — df_clean1)

Component	Value
Model type	Linear mixed model (lmer)
Formula	Carotenoids/Chla ~ Treatment × Light × Day + (1
Data	df_clean1
Optimizer	bobyqa
REML criterion	-200.2
Observations	144
Groups (Replicates)	16
Convergence	Yes

Random effects

Group	Effect	Variance	Std. Dev.
Replicates	Intercept	0.0003182	0.01784
Residual	—	0.0076710	0.08758

Fixed Effects (Linear Mixed Model)

Term	Estimate	SE	df	t value	p value	Sig
(Intercept)	0.61948	0.02681	58.60	23.106	<0.001	***
Treatment Teppeki	-0.06176	0.05212	104.51	-1.185	0.239	ns
Treatment Ortiva	-0.05124	0.05212	104.51	-0.983	0.328	ns
Treatment Basar	-0.12948	0.05212	104.51	-2.484	0.015	*
Light Weak	0.01652	0.03792	58.60	0.436	0.665	ns
Day Day 4	-0.05158	0.03576	108.00	-1.442	0.152	ns
Day Day 7	-0.09556	0.03576	108.00	-2.673	0.009	**
Teppeki × Light	-0.01652	0.07370	104.51	-0.224	0.823	ns
Ortiva × Light	-0.01652	0.07370	104.51	-0.224	0.823	ns
Basar × Light	-0.01652	0.07370	104.51	-0.224	0.823	ns
Teppeki × Day 4	0.07083	0.07151	108.00	0.990	0.324	ns
Ortiva × Day 4	-0.04735	0.07151	108.00	-0.662	0.509	ns
Basar × Day 4	-0.29461	0.07151	108.00	-4.120	<0.001	***
Teppeki × Day 7	0.21521	0.07151	108.00	3.009	0.003	**
Ortiva × Day 7	-0.15285	0.07151	108.00	-2.137	0.035	*
Basar × Day 7	-0.26306	0.07151	108.00	-3.679	<0.001	***
Light × Day 4	-0.18108	0.05057	108.00	-3.581	<0.001	***
Light × Day 7	-0.13303	0.05057	108.00	-2.631	0.010	**
Teppeki × Light × Day 4	-0.01446	0.10113	108.00	-0.143	0.887	ns
Ortiva × Light × Day 4	0.09988	0.10113	108.00	0.988	0.326	ns
Basar × Light × Day 4	0.13870	0.10113	108.00	1.371	0.173	ns
Teppeki × Light × Day 7	-0.20922	0.10113	108.00	-2.069	0.041	*
Ortiva × Light × Day 7	0.30338	0.10113	108.00	3.000	0.003	**
Basar × Light × Day 7	0.13158	0.10113	108.00	1.301	0.196	ns

Effect	Sum of Squares	Mean Square	Num DF	Den DF	F value	p value	Sig
Treatment	1.32801	0.44267	3	27.24	57.707	<0.001	***
Light	0.08346	0.08346	1	12.00	10.880	0.006	**

Effect	Sum of Squares	Mean Square	Num DF	Den DF	F value	p value	Sig
Day	0.85705	0.42852	2	108.00	55.863	<0.001	***
Treatment × Light	0.15283	0.05094	3	27.24	6.641	0.002	**
Treatment × Day	0.27360	0.04560	6	108.00	5.945	<0.001	***
Light × Day	0.07633	0.03817	2	108.00	4.975	0.009	**
Treatment × Light × Day	0.16339	0.02723	6	108.00	3.550	0.003	**

Table 11: GLMM results for Chl-b Chl-a^{-1} *in Chlorella vulgaris under three pesticide treatments*

Model Information

Category	Value
Response variable	Chl-b Chl-a^{-1}
Model type	Linear Mixed Model (LMM)
Distribution	Gaussian
Fixed effects	Treatment × Light × Day
Random effects	(1
Estimation method	REML
Test method	Satterthwaite
Optimizer	bobyqa
Observations	144
Groups	16 (Replicates)
Convergence	Yes
REML criterion	-333.9
Residual diagnostics	Scaled residuals approximately symmetric (min -4.09, max 2.13)

Random Effects

Group	Variance	Std. Dev.
Replicates (Intercept)	0.000095	0.009747
Residual	0.002524	0.050244

Fixed Effects (Summary Table)

Term	Estimate	SE	df	t value	p value	Sig
(Intercept)	1.54803	0.01530	59.81	101.17	<0.001	***
Treatment Teppeki	0.01457	0.02982	105.51	0.489	0.626	ns
Treatment Ortiva	-0.12471	0.02982	105.51	-4.183	<0.001	***

Term	Estimate	SE	df	t value	p value	Sig
Treatment Basar	-0.04333	0.02982	105.51	-1.453	0.149	ns
Light Weak	-0.00275	0.02164	59.81	-0.127	0.899	ns
Day Day 4	0.02312	0.02051	108.00	1.127	0.262	ns
Day Day 7	0.02242	0.02051	108.00	1.093	0.277	ns
Teppeki × Light	0.00275	0.04217	105.51	0.065	0.948	ns
Ortiva × Light	0.00275	0.04217	105.51	0.065	0.948	ns
Basar × Light	0.00275	0.04217	105.51	0.065	0.948	ns
Teppeki × Day 4	-0.00974	0.04102	108.00	-0.237	0.813	ns
Ortiva × Day 4	0.05284	0.04102	108.00	1.288	0.200	ns
Basar × Day 4	-0.08221	0.04102	108.00	-2.004	0.048	*
Teppeki × Day 7	-0.02703	0.04102	108.00	-0.659	0.511	ns
Ortiva × Day 7	0.10820	0.04102	108.00	2.638	0.010	**
Basar × Day 7	-0.04287	0.04102	108.00	-1.045	0.298	ns
Light × Day 4	0.00168	0.02901	108.00	0.058	0.954	ns
Light × Day 7	-0.03031	0.02901	108.00	-1.045	0.298	ns
Teppeki × Light × Day 4	-0.04641	0.05802	108.00	-0.800	0.426	ns
Ortiva × Light × Day 4	0.04895	0.05802	108.00	0.844	0.401	ns
Basar × Light × Day 4	0.05071	0.05802	108.00	0.874	0.384	ns
Teppeki × Light × Day 7	0.04472	0.05802	108.00	0.771	0.443	ns
Ortiva × Light × Day 7	0.06099	0.05802	108.00	1.051	0.296	ns
Basar × Light × Day 7	0.02016	0.05802	108.00	0.347	0.729	ns

Table 12: GLMM results for F_v/F_m *in Chlorella vulgaris under three pesticide treatments*

Model information

Component	Description
Model type	Linear mixed model (REML)
Response variable	Fv/Fm
Fixed effects	Pesticide * Light * Day
Random effects	(1
Estimation method	Satterthwaite’s t-tests
Optimizer	bobyqa
Observations	384
Groups	16 replicates

Random effects

Effect	Variance	Std. Dev.
Replicate (Intercept)	3.037×10^{-19}	5.511×10^{-10}
Residual	8.663×10^{-4}	2.943×10^{-2}

Fixed Effects Table (Complete Model)

Term	Estimate	Std. Error	df	t value	p value
(Intercept)	0.54075	0.01472	288	36.745	< 2e-16 ***
PesticideOrtiva	-0.33300	0.02081	288	-16.000	< 2e-16 ***
PesticideBasar	-0.15325	0.02081	288	-7.364	1.89e-12 ***
LightSP	0.01675	0.02081	288	0.805	0.421583
LightWC	0.02150	0.02081	288	1.033	0.302442
LightWP	0.00550	0.02081	288	0.264	0.791760
Day1	0.10075	0.02081	288	4.841	2.11e-06 ***
Day2	0.13950	0.02081	288	6.703	1.08e-10 ***
Day3	0.08475	0.02081	288	4.072	6.02e-05 ***
Day4	0.13925	0.02081	288	6.691	1.15e-10 ***
Day5	0.10000	0.02081	288	4.805	2.49e-06 ***
Day6	0.12525	0.02081	288	6.018	5.35e-09 ***
Day7	0.11050	0.02081	288	5.309	2.20e-07 ***
PesticideOrtiva:LightSP	-0.05875	0.02943	288	-1.996	0.046865 *
PesticideBasar:LightSP	-0.35425	0.02943	288	-12.036	< 2e-16 ***
PesticideOrtiva:LightWC	0.05325	0.02943	288	1.809	0.071459 .
PesticideBasar:LightWC	-0.01575	0.02943	288	-0.535	0.592978
PesticideOrtiva:LightWP	0.03450	0.02943	288	1.172	0.242096
PesticideBasar:LightWP	-0.34225	0.02943	288	-11.628	< 2e-16 ***
PesticideOrtiva:Day1	0.27400	0.02943	288	9.309	< 2e-16 ***
PesticideBasar:Day1	0.06750	0.02943	288	2.293	0.022546 *
PesticideOrtiva:Day2	0.29275	0.02943	288	9.946	< 2e-16 ***
PesticideBasar:Day2	0.14250	0.02943	288	4.842	2.10e-06 ***
PesticideOrtiva:Day3	0.29575	0.02943	288	10.048	< 2e-16 ***
PesticideBasar:Day3	0.11600	0.02943	288	3.941	0.000102 ***
PesticideOrtiva:Day4	0.27075	0.02943	288	9.199	< 2e-16 ***
PesticideBasar:Day4	0.08275	0.02943	288	2.812	0.005269 **
PesticideOrtiva:Day5	0.29700	0.02943	288	10.091	< 2e-16 ***
PesticideBasar:Day5	0.20750	0.02943	288	7.050	1.33e-11 ***
PesticideOrtiva:Day6	0.34925	0.02943	288	11.866	< 2e-16 ***
PesticideBasar:Day6	0.17275	0.02943	288	5.869	1.20e-08 ***

Term	Estimate	Std. Error	df	t value	p value
PesticideOrtiva:Day7	0.32425	0.02943	288	11.017	< 2e-16 ***
PesticideBasar:Day7	0.18975	0.02943	288	6.447	4.80e-10 ***
LightSP:Day1	-0.11200	0.02943	288	-3.805	0.000173 ***
LightWC:Day1	-0.00900	0.02943	288	-0.306	0.759990
LightWP:Day1	0.01300	0.02943	288	0.442	0.659046
LightSP:Day2	-0.00625	0.02943	288	-0.212	0.831984
LightWC:Day2	-0.01650	0.02943	288	-0.561	0.575503
LightWP:Day2	-0.01600	0.02943	288	-0.544	0.587126
LightSP:Day3	-0.02025	0.02943	288	-0.688	0.491997
LightWC:Day3	0.04450	0.02943	288	1.512	0.131647
LightWP:Day3	0.05125	0.02943	288	1.741	0.082704 .
LightSP:Day4	-0.01800	0.02943	288	-0.612	0.541306
LightWC:Day4	-0.02125	0.02943	288	-0.722	0.470886
LightWP:Day4	0.00700	0.02943	288	0.238	0.812180
LightSP:Day5	-0.04925	0.02943	288	-1.673	0.095350 .
LightWC:Day5	-0.00400	0.02943	288	-0.136	0.891992
LightWP:Day5	0.04125	0.02943	288	1.402	0.162138
LightSP:Day6	-0.02500	0.02943	288	-0.849	0.396364
LightWC:Day6	-0.03925	0.02943	288	-1.334	0.183402
LightWP:Day6	-0.01900	0.02943	288	-0.646	0.519088
LightSP:Day7	0.00325	0.02943	288	0.110	0.912152
LightWC:Day7	0.02400	0.02943	288	0.815	0.415503
LightWP:Day7	0.02025	0.02943	288	0.688	0.491997
PesticideOrtiva:LightSP:Day1	0.03875	0.04162	288	0.931	0.352656
PesticideBasar:LightSP:Day1	-0.04525	0.04162	288	-1.087	0.277894
PesticideOrtiva:LightWC:Day1	-0.06700	0.04162	288	-1.610	0.108569
PesticideBasar:LightWC:Day1	0.00050	0.04162	288	0.012	0.990424
PesticideOrtiva:LightWP:Day1	-0.07825	0.04162	288	-1.880	0.061127 .
PesticideBasar:LightWP:Day1	-0.15425	0.04162	288	-3.706	0.000253 ***
PesticideOrtiva:LightSP:Day2	0.04775	0.04162	288	1.147	0.252260
PesticideBasar:LightSP:Day2	-0.24125	0.04162	288	-5.796	1.78e-08 ***
PesticideOrtiva:LightWC:Day2	-0.01300	0.04162	288	-0.312	0.755023
PesticideBasar:LightWC:Day2	0.00225	0.04162	288	0.054	0.956928
PesticideOrtiva:LightWP:Day2	0.03550	0.04162	288	0.853	0.394437
PesticideBasar:LightWP:Day2	-0.21475	0.04162	288	-5.159	4.62e-07 ***

Term	Estimate	Std. Error	df	t value	p value
PesticideOrtiva:LightSP:Day3	0.05100	0.04162	288	1.225	0.221479
PesticideBasar:LightSP:Day3	0.34650	0.04162	288	8.325	3.45e-15 ***
PesticideOrtiva:LightWC:Day3	-0.02525	0.04162	288	-0.607	0.544579
PesticideBasar:LightWC:Day3	0.04375	0.04162	288	1.051	0.294103
PesticideOrtiva:LightWP:Day3	-0.04100	0.04162	288	-0.985	0.325445
PesticideBasar:LightWP:Day3	0.09625	0.04162	288	2.312	0.021462 *
PesticideOrtiva:LightSP:Day4	-0.00425	0.04162	288	-0.102	0.918744
PesticideBasar:LightSP:Day4	0.19675	0.04162	288	4.727	3.57e-06 ***
PesticideOrtiva:LightWC:Day4	-0.07050	0.04162	288	-1.694	0.091396 .
PesticideBasar:LightWC:Day4	0.07225	0.04162	288	1.736	0.083672 .
PesticideOrtiva:LightWP:Day4	-0.04525	0.04162	288	-1.087	0.277894
PesticideBasar:LightWP:Day4	0.25850	0.04162	288	6.210	1.84e-09 ***
PesticideOrtiva:LightSP:Day5	0.14425	0.04162	288	3.466	0.000610 ***
PesticideBasar:LightSP:Day5	0.08875	0.04162	288	2.132	0.033837 *
PesticideOrtiva:LightWC:Day5	0.00750	0.04162	288	0.180	0.857134
PesticideBasar:LightWC:Day5	-0.01100	0.04162	288	-0.264	0.791760
PesticideOrtiva:LightWP:Day5	-0.03150	0.04162	288	-0.757	0.449802
PesticideBasar:LightWP:Day5	0.23900	0.04162	288	5.742	2.37e-08 ***
PesticideOrtiva:LightSP:Day6	-0.02500	0.04162	288	-0.601	0.548567
PesticideBasar:LightSP:Day6	0.06625	0.04162	288	1.592	0.112564
PesticideOrtiva:LightWC:Day6	-0.04375	0.04162	288	-1.051	0.294103
PesticideBasar:LightWC:Day6	0.04975	0.04162	288	1.195	0.232981
PesticideOrtiva:LightWP:Day6	-0.03800	0.04162	288	-0.913	0.362039
PesticideBasar:LightWP:Day6	0.20200	0.04162	288	4.853	2.00e-06 ***
PesticideOrtiva:LightSP:Day7	-0.02050	0.04162	288	-0.493	0.622737
PesticideBasar:LightSP:Day7	0.02950	0.04162	288	0.709	0.479066
PesticideOrtiva:LightWC:Day7	-0.09850	0.04162	288	-2.366	0.018622 *
PesticideBasar:LightWC:Day7	-0.03375	0.04162	288	-0.811	0.418131
PesticideOrtiva:LightWP:Day7	-0.09050	0.04162	288	-2.174	0.030502 *
PesticideBasar:LightWP:Day7	0.14750	0.04162	288	3.544	0.000460 ***

Type III analysis of fixed effects (Satterthwaite's method)

Effect	Sum Sq	Mean Sq	NumDF	DenDF	F value	p value
Pesticide	1.9368	0.96838	2	288	1117.867	< 2.2e-16 ***
Light	1.2773	0.42576	3	288	491.484	< 2.2e-16 ***
Day	3.6405	0.52006	7	288	600.347	< 2.2e-16 ***

Effect	Sum Sq	Mean Sq	NumDF	DenDF	F value	p value
Pesticide:Light	1.6114	0.26856	6	288	310.024	< 2.2e-16 ***
Pesticide:Day	1.3581	0.09701	14	288	111.986	< 2.2e-16 ***
Light:Day	0.3815	0.01816	21	288	20.969	< 2.2e-16 ***
Pesticide:Light:Day	0.6995	0.01666	42	288	19.226	< 2.2e-16 ***

Table 13: GLMM results for F_v/F_m *in S. leopoliensis under three pesticide treatments*

Linear Mixed Model Summary (Fv/Fm)

Component	Statistic	Value
Model formula		Fv/Fm ~ Pesticide * Light * Day + (1
Data		PAM_SL
Method		REML (Satterthwaite's t-tests)
REML criterion		-883.2
Observations		384
Random effects groups		Replicate (n = 16)

Random effects

Effect	Variance	Std. Dev.
Replicate (Intercept)	8.224e-05	0.009069
Residual	1.663e-03	0.040780

Scaled residuals

Min	1Q	Median	3Q	Max
-4.1488	-0.4100	0.0282	0.3756	3.7300

Fixed Effects Table (Full Model)

Term	Estimate	Std. Error	df	t value	p value
(Intercept)	0.16025	0.02089	274.01	7.672	2.98e-13 ***
PesticideOrtiva	-0.01575	0.02884	276	-0.546	0.585370
PesticideBasar	0.13750	0.02884	276	4.768	3.01e-06 ***
LightSP	-0.02750	0.02954	274.01	-0.931	0.352703
LightWC	0.00300	0.02954	274.01	0.102	0.919182
LightWP	0.02550	0.02954	274.01	0.863	0.388762
Day1	-0.04350	0.02884	276	-1.509	0.132557
Day2	0.08625	0.02884	276	2.991	0.003031 **
Day3	0.00550	0.02884	276	0.191	0.848872
Day4	0.14850	0.02884	276	5.150	4.96e-07 ***

Term	Estimate	Std. Error	df	t value	p value
Day5	0.19750	0.02884	276	6.849	4.82e-11 ***
Day6	0.20725	0.02884	276	7.187	6.19e-12 ***
Day7	0.19400	0.02884	276	6.728	9.91e-11 ***
PesticideOrtiva:LightSP	-0.08625	0.04078	276	-2.115	0.035324 *
PesticideBasar:LightSP	-0.07075	0.04078	276	-1.735	0.083869 .
PesticideOrtiva:LightWC	0.03950	0.04078	276	0.969	0.333583
PesticideBasar:LightWC	0.00750	0.04078	276	0.184	0.854215
PesticideOrtiva:LightWP	-0.09275	0.04078	276	-2.274	0.023708 *
PesticideBasar:LightWP	-0.11950	0.04078	276	-2.930	0.003669 **
PesticideOrtiva:Day1	0.07200	0.04078	276	1.766	0.078571 .
PesticideBasar:Day1	0.10825	0.04078	276	2.655	0.008403 **
PesticideOrtiva:Day2	0.04900	0.04078	276	1.202	0.230557
PesticideBasar:Day2	-0.02225	0.04078	276	-0.546	0.585771
PesticideOrtiva:Day3	0.15325	0.04078	276	3.758	0.000209 ***
PesticideBasar:Day3	0.00650	0.04078	276	0.159	0.873476
PesticideOrtiva:Day4	-0.01175	0.04078	276	-0.288	0.773461
PesticideBasar:Day4	-0.16575	0.04078	276	-4.065	6.28e-05 ***
PesticideOrtiva:Day5	-0.02325	0.04078	276	-0.570	0.569049
PesticideBasar:Day5	-0.11125	0.04078	276	-2.728	0.006779 **
PesticideOrtiva:Day6	-0.02575	0.04078	276	-0.631	0.528274
PesticideBasar:Day6	-0.07675	0.04078	276	-1.882	0.060879 .
PesticideOrtiva:Day7	0.15675	0.04078	276	3.844	0.000150 ***
PesticideBasar:Day7	0.00675	0.04078	276	0.166	0.868653
LightSP:Day1	0.03075	0.04078	276	0.754	0.451461
LightWC:Day1	0.05400	0.04078	276	1.324	0.186536
LightWP:Day1	0.04800	0.04078	276	1.177	0.240187
LightSP:Day2	0.04350	0.04078	276	1.067	0.287036
LightWC:Day2	0.01350	0.04078	276	0.331	0.740860
LightWP:Day2	-0.00925	0.04078	276	-0.227	0.820725
LightSP:Day3	0.06950	0.04078	276	1.704	0.089454 .
LightWC:Day3	0.07900	0.04078	276	1.937	0.053736 .
LightWP:Day3	0.09125	0.04078	276	2.238	0.026043 *
LightSP:Day4	-0.00250	0.04078	276	-0.061	0.951161
LightWC:Day4	-0.03825	0.04078	276	-0.938	0.349081
LightWP:Day4	-0.08150	0.04078	276	-1.999	0.046638 *

Term	Estimate	Std. Error	df	t value	p value	
LightSP:Day5	-0.00150	0.04078	276	-0.037	0.970685	
LightWC:Day5	0.00500	0.04078	276	0.123	0.902505	
LightWP:Day5	-0.04425	0.04078	276	-1.085	0.278824	
LightSP:Day6	-0.11725	0.04078	276	-2.875	0.004352	**
LightWC:Day6	-0.05100	0.04078	276	-1.251	0.212131	
LightWP:Day6	-0.01650	0.04078	276	-0.405	0.686075	
LightSP:Day7	0.02550	0.04078	276	0.625	0.532283	
LightWC:Day7	-0.00550	0.04078	276	-0.135	0.892812	
LightWP:Day7	-0.04300	0.04078	276	-1.054	0.292601	
PesticideOrtiva:LightSP:Day1	-0.03700	0.05767	276	-0.642	0.521686	
PesticideBasar:LightSP:Day1	-0.12250	0.05767	276	-2.124	0.034550	*
PesticideOrtiva:LightWC:Day1	-0.07000	0.05767	276	-1.214	0.225870	
PesticideBasar:LightWC:Day1	-0.01100	0.05767	276	-0.191	0.848872	
PesticideOrtiva:LightWP:Day1	-0.11700	0.05767	276	-2.029	0.043444	*
PesticideBasar:LightWP:Day1	-0.08100	0.05767	276	-1.405	0.161290	
PesticideOrtiva:LightSP:Day2	-0.17800	0.05767	276	-3.086	0.002232	**
PesticideBasar:LightSP:Day2	-0.06350	0.05767	276	-1.101	0.271825	
PesticideOrtiva:LightWC:Day2	-0.03800	0.05767	276	-0.659	0.510504	
PesticideBasar:LightWC:Day2	-0.07275	0.05767	276	-1.261	0.208207	
PesticideOrtiva:LightWP:Day2	-0.17250	0.05767	276	-2.991	0.003031	**
PesticideBasar:LightWP:Day2	0.00550	0.05767	276	0.095	0.924092	
PesticideOrtiva:LightSP:Day3	-0.21950	0.05767	276	-3.806	0.000174	***
PesticideBasar:LightSP:Day3	-0.05050	0.05767	276	-0.876	0.381979	
PesticideOrtiva:LightWC:Day3	-0.16125	0.05767	276	-2.796	0.005537	**
PesticideBasar:LightWC:Day3	-0.14750	0.05767	276	-2.558	0.011074	*
PesticideOrtiva:LightWP:Day3	-0.28825	0.05767	276	-4.998	1.03e-06	***
PesticideBasar:LightWP:Day3	-0.09275	0.05767	276	-1.608	0.108922	
PesticideOrtiva:LightSP:Day4	-0.09675	0.05767	276	-1.678	0.094554	.
PesticideBasar:LightSP:Day4	0.06700	0.05767	276	1.162	0.246337	
PesticideOrtiva:LightWC:Day4	-0.00800	0.05767	276	-0.139	0.889775	
PesticideBasar:LightWC:Day4	0.05175	0.05767	276	0.897	0.370326	
PesticideOrtiva:LightWP:Day4	-0.02625	0.05767	276	-0.455	0.649347	
PesticideBasar:LightWP:Day4	0.09650	0.05767	276	1.673	0.095405	.
PesticideOrtiva:LightSP:Day5	0.00350	0.05767	276	0.061	0.951651	
PesticideBasar:LightSP:Day5	0.00050	0.05767	276	0.009	0.993089	

Term	Estimate	Std. Error	df	t value	p value
PesticideOrtiva:LightWC:Day5	-0.06075	0.05767	276	-1.053	0.293085
PesticideBasar:LightWC:Day5	-0.02525	0.05767	276	-0.438	0.661854
PesticideOrtiva:LightWP:Day5	0.02750	0.05767	276	0.477	0.633852
PesticideBasar:LightWP:Day5	0.01825	0.05767	276	0.316	0.751901
PesticideOrtiva:LightSP:Day6	0.12150	0.05767	276	2.107	0.036040 *
PesticideBasar:LightSP:Day6	0.07500	0.05767	276	1.300	0.194522
PesticideOrtiva:LightWC:Day6	-0.00800	0.05767	276	-0.139	0.889775
PesticideBasar:LightWC:Day6	-0.03350	0.05767	276	-0.581	0.561796
PesticideOrtiva:LightWP:Day6	0.02000	0.05767	276	0.347	0.729011
PesticideBasar:LightWP:Day6	0.05650	0.05767	276	0.980	0.328096
PesticideOrtiva:LightSP:Day7	-0.18325	0.05767	276	-3.177	0.001654 **
PesticideBasar:LightSP:Day7	-0.12300	0.05767	276	-2.133	0.033825 *
PesticideOrtiva:LightWC:Day7	-0.20900	0.05767	276	-3.624	0.000345 ***
PesticideBasar:LightWC:Day7	-0.14725	0.05767	276	-2.553	0.011210 *
PesticideOrtiva:LightWP:Day7	-0.10675	0.05767	276	-1.851	0.065236 .
PesticideBasar:LightWP:Day7	0.03250	0.05767	276	0.564	0.573525

Type III ANOVA Table (Satterthwaite's method)

Effect	Sum Sq	Mean Sq	NumDF	DenDF	F value	p value
Pesticide	0.68787	0.34393	2	276	206.8172	< 2.2e-16 ***
Light	0.34847	0.11616	3	12	69.8487	7.232e-08 ***
Day	1.49790	0.21399	7	276	128.6756	< 2.2e-16 ***
Pesticide:Light	0.39298	0.06550	6	276	39.3850	< 2.2e-16 ***
Pesticide:Day	0.22093	0.01578	14	276	9.4892	< 2.2e-16 ***
Light:Day	0.15610	0.00743	21	276	4.4700	2.087e-09 ***
Pesticide:Light:Day	0.27877	0.00664	42	276	3.9913	1.819e-12 ***

*Table 14: GLMM results for **RLC** in Chlorella vulgaris under three pesticide treatments*

Linear Mixed Model Summary (α)

Component	Statistic	Value
Model formula		α ~ Pesticide * Light * Day + (1
Data		RLC_CV
Method		REML (Satterthwaite's t-tests)
REML criterion		-412.2
Observations		144

Component	Statistic	Value
Random-effect groups		Replicate (n = 16)

Random effects

Effect	Variance	Std. Dev.
Replicate (Intercept)	0.000000	0.00000
Residual	0.001353	0.03679

Scaled residuals

Min	1Q	Median	3Q	Max
-2.38526	-0.37886	0.02718	0.33638	2.69786

Fixed Effects Table (Full Model)

Term	Estimate	Std. Error	df	t value	p value
(Intercept)	1.988e-01	1.062e-02	120	18.715	< 2e-16 ***
PesticideTeppeki	2.750e-02	2.124e-02	120	1.295	0.19790
PesticideOrtiva	-6.025e-02	2.124e-02	120	-2.837	0.00535 **
PesticideBasar	-6.475e-02	2.124e-02	120	-3.049	0.00283 **
LightWeak	-2.145e-16	1.502e-02	120	0.000	1.00000
Day4	7.233e-02	1.502e-02	120	4.816	4.32e-06 ***
Day7	6.717e-02	1.502e-02	120	4.472	1.77e-05 ***
PesticideTeppeki:LightWeak	7.300e-16	3.004e-02	120	0.000	1.00000
PesticideOrtiva:LightWeak	4.993e-16	3.004e-02	120	0.000	1.00000
PesticideBasar:LightWeak	1.878e-16	3.004e-02	120	0.000	1.00000
PesticideTeppeki:Day4	-8.833e-03	3.004e-02	120	-0.294	0.76921
PesticideOrtiva:Day4	1.217e-02	3.004e-02	120	0.405	0.68616
PesticideBasar:Day4	-2.033e-02	3.004e-02	120	-0.677	0.49975
PesticideTeppeki:Day7	-1.042e-02	3.004e-02	120	-0.347	0.72936
PesticideOrtiva:Day7	1.533e-02	3.004e-02	120	0.510	0.61066
PesticideBasar:Day7	-4.417e-02	3.004e-02	120	-1.470	0.14408
LightWeak:Day4	-1.017e-02	2.124e-02	120	-0.479	0.63305
LightWeak:Day7	8.500e-03	2.124e-02	120	0.400	0.68973
PesticideTeppeki:LightWeak:Day4	2.917e-03	4.248e-02	120	0.069	0.94537
PesticideOrtiva:LightWeak:Day4	3.417e-02	4.248e-02	120	0.804	0.42281
PesticideBasar:LightWeak:Day4	-5.283e-02	4.248e-02	120	-1.244	0.21602
PesticideTeppeki:LightWeak:Day7	-8.250e-03	4.248e-02	120	-0.194	0.84634
PesticideOrtiva:LightWeak:Day7	4.875e-02	4.248e-02	120	1.148	0.25341

Term	Estimate	Std. Error	df	t value	p value
PesticideBasar:LightWeak:Day7	1.150e-02	4.248e-02	120	0.271	0.78707

Type III analysis of fixed effects (Satterthwaite's method)

Effect	Sum Sq	Mean Sq	NumDF	DenDF	F value	p value
Pesticide	0.202727	0.067576	3	120	49.9306	< 2.2e-16 ***
Light	0.000175	0.000175	1	120	0.1293	0.7198
Day	0.107687	0.053843	2	120	39.7840	5.564e-14 ***
Pesticide:Light	0.005595	0.001865	3	120	1.3781	0.2528
Pesticide:Day	0.017160	0.002860	6	120	2.1132	0.0566 .
Light:Day	0.006172	0.003086	2	120	2.2803	0.1067
Pesticide:Light:Day	0.006509	0.001085	6	120	0.8015	0.5706

Linear Mixed Model Summary (ETRmax)

Component	Statistic	Value
Model formula		ETRmax ~ Pesticide * Light * Day + (1
Data		RLC_CV
Method		REML (Satterthwaite's t-tests)
REML criterion		958.7
Observations		144
Random-effect groups		Replicate (n = 16)

Random effects

Effect	Variance	Std. Dev.
Replicate (Intercept)	0.000	0.000
Residual	123.8	11.13

Scaled residuals

Min	1Q	Median	3Q	Max
-2.11544	-0.33473	-0.06665	0.32405	2.79986

Fixed Effects Table (ETRmax)

Term	Estimate	Std. Error	df	t value	p value
(Intercept)	2.834e+01	3.213e+00	120	8.822	1.08e-14 ***
PesticideTeppeki	3.783e+00	6.425e+00	120	0.589	0.557075
PesticideOrtiva	-1.817e+01	6.425e+00	120	-2.827	0.005499 **
PesticideBasar	-6.092e+00	6.425e+00	120	-0.948	0.344978
LightWeak	-5.380e-13	4.543e+00	120	0.000	1.000000
Day4	2.819e+01	4.543e+00	120	6.205	8.06e-09 ***

Term	Estimate	Std. Error	df	t value	p value
Day7	1.593e+01	4.543e+00	120	3.507	0.000638 ***
PesticideTeppeki:LightWeak	5.736e-13	9.086e+00	120	0.000	1.000000
PesticideOrtiva:LightWeak	5.952e-13	9.086e+00	120	0.000	1.000000
PesticideBasar:LightWeak	5.442e-13	9.086e+00	120	0.000	1.000000
PesticideTeppeki:Day4	-7.617e+00	9.086e+00	120	-0.838	0.403557
PesticideOrtiva:Day4	2.168e+01	9.086e+00	120	2.386	0.018580 *
PesticideBasar:Day4	-1.877e+01	9.086e+00	120	-2.065	0.041043 *
PesticideTeppeki:Day7	-2.933e+00	9.086e+00	120	-0.323	0.747388
PesticideOrtiva:Day7	3.052e+01	9.086e+00	120	3.358	0.001051 **
PesticideBasar:Day7	6.417e+00	9.086e+00	120	0.706	0.481443
LightWeak:Day4	-4.225e+00	6.425e+00	120	-0.658	0.512066
LightWeak:Day7	1.775e+00	6.425e+00	120	0.276	0.782822
PesticideTeppeki:LightWeak:Day4	5.150e+00	1.285e+01	120	0.401	0.689299
PesticideOrtiva:LightWeak:Day4	3.100e+00	1.285e+01	120	0.241	0.809778
PesticideBasar:LightWeak:Day4	-2.000e-01	1.285e+01	120	-0.016	0.987608
PesticideTeppeki:LightWeak:Day7	1.725e+00	1.285e+01	120	0.134	0.893438
PesticideOrtiva:LightWeak:Day7	5.975e+00	1.285e+01	120	0.465	0.642791
PesticideBasar:LightWeak:Day7	-3.900e+00	1.285e+01	120	-0.303	0.762035

Type III analysis of fixed effects (Satterthwaite's method) — ETRmax

Effect	Sum Sq	Mean Sq	NumDF	DenDF	F value	p value
Pesticide	2603.2	867.7	3	120	7.0067	0.0002208 ***
Light	0.8	0.8	1	120	0.0068	0.9344805
Day	17110.9	8555.4	2	120	69.0824	< 2.2e-16 ***
Pesticide:Light	82.0	27.3	3	120	0.2208	0.8818256
Pesticide:Day	6103.6	1017.3	6	120	8.2141	1.876e-07 ***
Light:Day	117.4	58.7	2	120	0.4741	0.6235805
Pesticide:Light:Day	71.7	11.9	6	120	0.0965	0.9966131

Linear Mixed Model Summary (IK)

Component	Statistic	Value
Model formula		IK ~ Pesticide * Light * Day + (1
Data		RLC_CV
Method		REML (Satterthwaite's t-tests)
REML criterion		1252.9
Observations		144

Component	Statistic	Value
Random-effect groups	Replicate (n = 16)	

Random effects

Effect	Variance	Std. Dev.
Replicate (Intercept)	0.000	0.000
Residual	1438.0	37.92

Scaled residuals

Min	1Q	Median	3Q	Max
-2.23337	-0.44598	-0.00004	0.40329	2.53644

Fixed Effects Table (IK)

Term	Estimate	Std. Error	df	t value	p value
(Intercept)	1.245e+02	1.095e+01	120	11.373	< 2e-16 ***
PesticideTeppeki	1.957e+01	2.189e+01	120	0.894	0.373219
PesticideOrtiva	-4.561e+01	2.189e+01	120	-2.084	0.039325 *
PesticideBasar	4.159e+01	2.189e+01	120	1.900	0.059847 .
LightWeak	3.111e-12	1.548e+01	120	0.000	1.000000
Day4	8.493e+01	1.548e+01	120	5.486	2.32e-07 ***
Day7	4.086e+01	1.548e+01	120	2.639	0.009411 **
PesticideTeppeki:LightWeak	-3.302e-12	3.096e+01	120	0.000	1.000000
PesticideOrtiva:LightWeak	3.571e-03	3.096e+01	120	0.000	0.999908
PesticideBasar:LightWeak	-3.272e-12	3.096e+01	120	0.000	1.000000
PesticideTeppeki:Day4	-4.623e+01	3.096e+01	120	-1.493	0.138040
PesticideOrtiva:Day4	1.061e+02	3.096e+01	120	3.427	0.000836 ***
PesticideBasar:Day4	-6.928e+01	3.096e+01	120	-2.238	0.027092 *
PesticideTeppeki:Day7	-2.566e+01	3.096e+01	120	-0.829	0.408932
PesticideOrtiva:Day7	1.406e+02	3.096e+01	120	4.540	1.34e-05 ***
PesticideBasar:Day7	7.849e+01	3.096e+01	120	2.535	0.012517 *
LightWeak:Day4	-1.013e+01	2.189e+01	120	-0.463	0.644556
LightWeak:Day7	2.678e+00	2.189e+01	120	0.122	0.902843
PesticideTeppeki:LightWeak:Day4	1.715e+01	4.378e+01	120	0.392	0.695972
PesticideOrtiva:LightWeak:Day4	-2.125e+01	4.378e+01	120	-0.485	0.628258
PesticideBasar:LightWeak:Day4	5.085e+01	4.378e+01	120	1.161	0.247782
PesticideTeppeki:LightWeak:Day7	9.722e+00	4.378e+01	120	0.222	0.824653
PesticideOrtiva:LightWeak:Day7	-3.248e+01	4.378e+01	120	-0.742	0.459647

Term	Estimate	Std. Error	df	t value	p value
PesticideBasar:LightWeak:Day7	-4.733e+01	4.378e+01	120	-1.081	0.281881

Type III analysis of fixed effects (Satterthwaite's method) — IK

Effect	Sum Sq	Mean Sq	NumDF	DenDF	F value	p value
Pesticide	46193	15398	3	120	10.7097	2.738e-06 ***
Light	564	564	1	120	0.3925	0.5322
Day	174589	87295	2	120	60.7172	< 2.2e-16 ***
Pesticide:Light	2353	784	3	120	0.5456	0.6521
Pesticide:Day	92054	15342	6	120	10.6713	1.755e-09 ***
Light:Day	1574	787	2	120	0.5474	0.5799
Pesticide:Light:Day	8722	1454	6	120	1.0111	0.4214

*Table 15: GLMM results for **RLC** in S. leopoliensis under three pesticide treatments*

Linear Mixed Model Summary (α)

Component	Statistic	Value
Model formula		α ~ Pesticide * Light * Day + (1
Data		RLC_SL
Method		REML (Satterthwaite's t-tests)
REML criterion		-617.7
Observations		144
Random-effect groups		Replicate (n = 16)

Random effects

Effect	Variance	Std. Dev.
Replicate (Intercept)	3.227e-19	5.681e-10
Residual	2.442e-04	0.01563

Scaled residuals

Min	1Q	Median	3Q	Max
-1.96775	-0.51193	-0.04799	0.40395	2.63966

Fixed Effects Table (α)

Term	Estimate	Std. Error	df	t value	p value
(Intercept)	4.400e-02	4.511e-03	120	9.754	< 2e-16 ***
PesticideTeppeki	1.175e-02	9.022e-03	120	1.302	0.195296
PesticideOrtiva	7.250e-02	9.022e-03	120	8.036	7.31e-13 ***
PesticideBasar	-7.250e-03	9.022e-03	120	-0.804	0.423235

Term	Estimate	Std. Error	df	t value	p value
LightWeak	-2.518e-16	6.380e-03	120	0.000	1.000000
Day4	9.908e-02	6.380e-03	120	15.531	< 2e-16 ***
Day7	1.027e-01	6.380e-03	120	16.106	< 2e-16 ***
PesticideTeppeki:LightWeak	1.924e-16	1.276e-02	120	0.000	1.000000
PesticideOrtiva:LightWeak	7.191e-17	1.276e-02	120	0.000	1.000000
PesticideBasar:LightWeak	1.084e-16	1.276e-02	120	0.000	1.000000
PesticideTeppeki:Day4	-2.483e-02	1.276e-02	120	-1.946	0.053959 .
PesticideOrtiva:Day4	-9.183e-02	1.276e-02	120	-7.197	5.80e-11 ***
PesticideBasar:Day4	-4.833e-02	1.276e-02	120	-3.788	0.000239 ***
PesticideTeppeki:Day7	-2.925e-02	1.276e-02	120	-2.292	0.023623 *
PesticideOrtiva:Day7	-8.650e-02	1.276e-02	120	-6.779	4.81e-10 ***
PesticideBasar:Day7	-3.650e-02	1.276e-02	120	-2.861	0.004989 **
LightWeak:Day4	-1.333e-02	9.022e-03	120	-1.478	0.142074
LightWeak:Day7	-2.625e-02	9.022e-03	120	-2.909	0.004316 **
PesticideTeppeki:LightWeak:Day4	1.083e-02	1.804e-02	120	0.600	0.549394
PesticideOrtiva:LightWeak:Day4	3.583e-03	1.804e-02	120	0.199	0.842925
PesticideBasar:LightWeak:Day4	7.333e-03	1.804e-02	120	0.406	0.685171
PesticideTeppeki:LightWeak:Day7	8.250e-03	1.804e-02	120	0.457	0.648352
PesticideOrtiva:LightWeak:Day7	-2.000e-03	1.804e-02	120	-0.111	0.911931
PesticideBasar:LightWeak:Day7	3.825e-02	1.804e-02	120	2.120	0.036087 *

Type III analysis of fixed effects (α)

Effect	Sum Sq	Mean Sq	NumDF	DenDF	F value	p value
Pesticide	0.021956	0.007319	3	120	29.9702	1.562e-14 ***
Light	0.001696	0.001696	1	120	6.9445	0.009518 **
Day	0.079071	0.039535	2	120	161.8951	< 2.2e-16 ***
Pesticide:Light	0.001142	0.000381	3	120	1.5588	0.202975
Pesticide:Day	0.034614	0.005769	6	120	23.6236	< 2.2e-16 ***
Light:Day	0.001099	0.000549	2	120	2.2497	0.109856
Pesticide:Light:Day	0.001478	0.000246	6	120	1.0090	0.422762

Linear Mixed Model Summary (ETRmax, RLC_SL)

Component	Value
Model	ETRmax ~ Pesticide × Light × Day + (1
Data	RLC_SL

Component	Value
Method	REML (Satterthwaite t-tests)
Observations	144
Replicates	16
REML criterion	744.6

Random Effects

Group	Effect	Variance	Std. Dev.
Replicate	Intercept	0.00	0.00
Residual	—	20.79	4.56

Residual Diagnostics (Scaled)

Statistic	Value
Min	-3.2677
1Q	-0.2394
Median	0.0018
3Q	0.2650
Max	4.9126

Fixed Effects Table (ETRmax)

Term	Estimate	Std. Error	df	t value	p value
(Intercept)	3.792e+00	1.316e+00	120	2.881	0.00470 **
PesticideTeppeki	-1.142e+00	2.633e+00	120	-0.434	0.66531
PesticideOrtiva	1.338e+01	2.633e+00	120	5.084	1.38e-06 ***
PesticideBasar	-4.167e-02	2.633e+00	120	-0.016	0.98740
LightWeak	-2.890e-14	1.862e+00	120	0.000	1.00000
Day4	2.220e+01	1.862e+00	120	11.926	< 2e-16 ***
Day7	2.971e+01	1.862e+00	120	15.959	< 2e-16 ***
PesticideTeppeki:LightWeak	5.263e-14	3.723e+00	120	0.000	1.00000
PesticideOrtiva:LightWeak	-7.032e-15	3.723e+00	120	0.000	1.00000
PesticideBasar:LightWeak	2.892e-14	3.723e+00	120	0.000	1.00000
PesticideTeppeki:Day4	-2.250e-01	3.723e+00	120	-0.060	0.95191
PesticideOrtiva:Day4	-1.553e+01	3.723e+00	120	-4.170	5.78e-05 ***
PesticideBasar:Day4	6.250e-01	3.723e+00	120	0.168	0.86696
PesticideTeppeki:Day7	-1.053e+01	3.723e+00	120	-2.829	0.00547 **
PesticideOrtiva:Day7	-2.311e+01	3.723e+00	120	-6.207	8.00e-09 ***
PesticideBasar:Day7	-1.688e+01	3.723e+00	120	-4.535	1.38e-05 ***

Term	Estimate	Std. Error	df	t value	p value
LightWeak:Day4	-4.967e+00	2.633e+00	120	-1.887	0.06163 .
LightWeak:Day7	-1.786e+01	2.633e+00	120	-6.784	4.71e-10 ***
PesticideTeppeki:LightWeak:Day4	4.417e-01	5.265e+00	120	0.084	0.93329
PesticideOrtiva:LightWeak:Day4	8.717e+00	5.265e+00	120	1.656	0.10043
PesticideBasar:LightWeak:Day4	-7.833e+00	5.265e+00	120	-1.488	0.13943
PesticideTeppeki:LightWeak:Day7	9.383e+00	5.265e+00	120	1.782	0.07725 .
PesticideOrtiva:LightWeak:Day7	1.358e+01	5.265e+00	120	2.580	0.01109 *
PesticideBasar:LightWeak:Day7	8.808e+00	5.265e+00	120	1.673	0.09694 .

Type III analysis of fixed effects (Satterthwaite's method) – ETRmax ~ Pesticide × Light × Day (RLC_SL)

Effect	Sum Sq	Mean Sq	NumDF	DenDF	F value	p-value
Pesticide	1261.4	420.47	3	120	20.2234	1.127e-10 ***
Light	677.4	677.45	1	120	32.5834	8.412e-08 ***
Day	5401.4	2700.69	2	120	129.8958	< 2.2e-16 ***
Pesticide:Light	274.8	91.59	3	120	4.4054	0.005614 **
Pesticide:Day	1157.2	192.87	6	120	9.2767	2.396e-08 ***
Light:Day	472.5	236.25	2	120	11.3629	3.025e-05 ***
Pesticide:Light:Day	363.4	60.57	6	120	2.9134	0.010931 *

Linear mixed model (IK ~ Pesticide × Light × Day + (1|Replicate), RLC_SL)

Model information

Component	Value
Formula	IK ~ Pesticide × Light × Day + (1
Data	RLC_SL
Optimizer	bobyqa
REML criterion at convergence	1104.6
Number of observations	144
Number of groups (Replicate)	16

Random effects

Group	Term	Variance	Std. Dev.
Replicate	Intercept	0.0	0.00
Residual	—	417.8	20.44

Fixed effects (IK ~ Pesticide × Light × Day, RLC_SL)

Term	Estimate	Std. Error	df	t value	p-value
(Intercept)	88.490	5.900	120	14.998	< 2e-16 ***
Pesticide Teppeki	-36.140	11.800	120	-3.063	0.002708 **
Pesticide Ortiva	59.560	11.800	120	5.047	1.62e-06 ***
Pesticide Basar	18.510	11.800	120	1.568	0.119413
Light Weak	0.000	8.344	120	0.000	1.000000
Day 4	92.750	8.344	120	11.115	< 2e-16 ***
Day 7	79.270	8.344	120	9.501	2.67e-16 ***
Teppeki:Weak	0.000	16.690	120	0.000	1.000000
Ortiva:Weak	0.000	16.690	120	0.000	1.000000
Basar:Weak	0.000	16.690	120	0.000	1.000000
Teppeki:Day4	46.650	16.690	120	2.795	0.006039 **
Ortiva:Day4	-47.850	16.690	120	-2.867	0.004892 **
Basar:Day4	-45.020	16.690	120	-2.698	0.007982 **
Teppeki:Day7	38.930	16.690	120	2.332	0.021343 *
Ortiva:Day7	-63.270	16.690	120	-3.792	0.000236 ***
Basar:Day7	-40.550	16.690	120	-2.430	0.016587 *
Weak:Day4	-22.110	11.800	120	-1.874	0.063431 .
Weak:Day7	-37.300	11.800	120	-3.161	0.001992 **
Teppeki:Weak:Day4	-10.820	23.600	120	-0.458	0.647556
Ortiva:Weak:Day4	1.758	23.600	120	0.075	0.940735
Basar:Weak:Day4	-9.942	23.600	120	-0.421	0.674335
Teppeki:Weak:Day7	-11.980	23.600	120	-0.507	0.612810
Ortiva:Weak:Day7	38.000	23.600	120	1.610	0.110004
Basar:Weak:Day7	-38.000	23.600	120	-1.610	0.110004

Type III analysis of fixed effects (Satterthwaite's method) – IK ~ Pesticide × Light × Day (RLC_SL)

Effect	Sum Sq	Mean Sq	NumDF	DenDF	F value	p-value
Pesticide	31293	10431	3	120	24.9688	1.269e-12 ***
Light	14430	14430	1	120	34.5418	3.831e-08 ***
Day	90250	45125	2	120	108.0178	< 2.2e-16 ***
Pesticide:Light	2826	942	3	120	2.2547	0.0855137 .

Effect	Sum Sq	Mean Sq	NumDF	DenDF	F value	p-value
Pesticide:Day	31393	5232	6	120	12.5243	6.308e-11 ***
Light:Day	8081	4041	2	120	9.6725	0.0001275 ***
Pesticide:Light:Day	3507	584	6	120	1.3990	0.2205362

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