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Comparative Responses of Protein and Chlorophyll *a* of Freshwater Microalgae Toward Three Organophosphorus Pesticides

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ABSTRACT

Organophosphorus pesticides such as Dimethoate, Malathion, and Tamaron are among the most commonly used insecticides in agriculture in Egypt. The toxic effects of these pesticides on aquatic ecosystem organisms were studied using several freshwater microalgae species, including *Chlamydomonas globosa*, *Chlorella vulgaris*, *Scenedesmus obliquus*, *Microcystis aeruginosa*, *Nostoc punctiforme*, *Gomphonema olivaceum*, *Navicula submuralis*, and *Nitzschia* sp., along with the standard toxicity microalga, *Raphidocelis subcapitata*. The lethal concentration (LC50) and linear regression analysis were used to investigate the relationship between pesticide concentration, protein content, and chlorophyll a (Chl a) in the freshwater microalgae. The relative sensitivity of Chl a and protein content varied depending on the pesticide concentration and the specific microalgal species tested. The total protein content of all tested diatom species was more sensitive to Dimethoate than their Chl a levels. In contrast, the protein content of other algae species, particularly the blue-green algae *M. aeruginosa* and *N. punctiforme*, was more tolerant to Dimethoate than Chl a. For Malathion, Chl a in most of the studied microalgae was more resistant than the protein content, whereas for Tamaron, protein content was more resistant than Chl a. Both Chl a and protein content can serve as relevant indicators for assessing pesticide effects on microalgae, but they may provide distinct insights. The toxicity of pesticides to microalgae cannot be fully determined by protein content alone. Future research on pesticides should aim to clarify their specific impacts on algae and explore a more comprehensive understanding of their effects.

INTRODUCTION

Agriculture is the backbone of the national economy, and pesticides have been widely used to increase agricultural productivity and food supply **(Sexton *et al*., 2007)**. Most pesticides are toxic, and if not properly managed, they can contaminate soil and water, posing a threat to wildlife **(Li *et al*., 2014; Maksymiv, 2015; Khan *et al*., 2016; Pan *et al*., 2024)**. In recent years, the worldwide consumption of pesticides has been about two million tons per year **(De *et al*., 2014)**. Tragically, most pesticides are non-

selective, however, they should be toxic only to the target organism, biodegradable and to some extent environmentally friendly **(Rosell *et al*., 2008)**.

Organophosphorus pesticides (e.g. Dimethoate, Malathion, and Tamaron**)** are hazardous organic compounds that act in insects' and mammals' nervous systems as acetylcholinesterase (AChE) inhibitors. Because the organophosphorylated enzyme is highly persistent in many situations, recovery from toxicity can be prolonged **(WHO 1986; IPCS, 1994)**. The organophosphorous pesticides have been determined to move to groundwater due to non-point source applications of soil-applied pesticides **(Schuette *et al*., 2006)**. Organophosphate pesticides have increased significantly in recent decades due to their low persistence in the environment and their powerful effects **(Vagi *et al*., 2005; Epa, 2006)**. According to the German Federal Office for Consumer Protection and Food Safety **(BVL, 2019)** and the European Food Safety Authority **(EFSA, 2019)**, the EU Commission has restricted the use of plant protection products containing Dimethoate **(EU, 2019a, b)**. However, many non-EU countries still continue to use these products.

Algae are the primary producers in aquatic ecosystems and the undesirable side-effects of contaminated ecosystems may affect the entire food chain. Microalgae have demonstrated sensitivity to a wide range of contaminants, such as insecticides **(Sabater & Carrasco 2001; Gómez de Barreda Ferraz *et al*., 2004; Dupraz *et al*., 2019)**, herbicides **(Rioboo *et al*., 2002; Sabater *et al*., 2002; Moro *et al*., 2012; Stenstrom *et al*., 2021)** and heavy metals **(Atici *et al*., 2008; Mayer-Pinto *et al*., 2011)**. Aquatic environments are often contaminated with different insecticides mainly from agriculture runoff **(Adrislaine *et al*, 2017)**. However, some studies have found increased algal biomass in aquatic habitats after pesticide exposure **(Brock *et al*., 2000)**. Several researchers have studied the toxicity and differential sensitivity of several pesticides, discovering that the toxicity and behavior of fungicides fluctuate significantly amongst various sediments and waterways **(Jianyi *et al*., 2006; Adam *et al*., 2010; Salwa *et al*., 2012; Simon *et al*., 2017)**. Risk assessment of pesticides has been based on direct toxic effects on aquatic organisms **(Bessa *et al*., 2016; Mavrogenis *et al*., 2023)**. It is well known that atrazine pesticide exposure dramatically lowers the synthesis of proteins in various species of diatoms and causes fat accumulation in cultured diatoms **(Weiner *et al*., 2007)**. It has also been observed that pesticides inhibit the synthesis of amino acids, such as valine and isoleucine, disrupting the biosynthesis of algae proteins **(Rimet *et al*., 1999; Singhal *et al*., 2022)**.

Chlorophyll *a* measurement is one of the most widely used parameters to assess the effects of pesticides on algae growth **(Debenest *et al*., 2024)**. To shed light on the underlying mechanisms at work, the present study investigates the toxicity effect of three organophosphorus pesticides; Malathion, Dimethoate and Tamaron on the biochemical contents of some selected microalgae species to discover if protein content alone can be used as a direct indicator of pesticide toxicity as chlorophyll *a* content.

MATERIALS AND METHODS

1- Test organism and culture conditions

The microalgal species used in this study are among the most common in freshwater ecosystems. The cyanobacteria species include *Microcystis aeruginosa* (Kützing) Kützing and *Nostoc punctiforme* (Hariot). The green microalgae species are *Chlamydomonas globosa* (J.W. Snow), *Chlorella vulgaris* (Beyerinck), and *Scenedesmus obliquus* (Turpin) Kützing. The diatom species are *Gomphonema olivaceum* (Hornemann) Ehrenberg, *Navicula submuralis* Hustedt, and *Nitzschia* sp. Additionally, the toxicity standard microalga *Raphidocelis subcapitata* (Korshikov) Nygaard, Komárek, J. Kristiansen & O.M. Skulberg (1987), previously known as *Pseudokirchneriella subcapitata* (Korshikov) F. Hindák and *Selenastrum capricornutum* Printz (1914), was also used. Freshwater samples were collected from the Damietta Branch of the River Nile using the Streak Plating method **(Stein, 1973)**. The Algal Assay Medium (AAM) **(Miller *et al.*, 1978)** was used for culturing the test species, and for the diatom species, 10 mg/L of Si was added to the culture medium.

2- Pesticides and experimental design

Three organophosphorus pesticides—Dimethoate (40%), Malathion (57%), and Tamaron (60%)—were widely used as insecticides in agriculture (Table 1). Large amounts of these pesticides were discharged into aquatic ecosystems, posing potential risks. The impacts of these insecticides were studied using some of the most common freshwater microalgae species. Toxicity tests followed the protocols of the **USEPA (1996)** and **OECD (2002)**. A preliminary test was conducted using the standard bio-test microalga *Raphidocelis subcapitata* to determine the effective concentration range. The pesticide concentrations used were as follows: Dimethoate (20, 40, 60, 80, and 100mg/ L), Malathion (13, 16, 18, 26, 32, 40, 60, 80, and 100mg/ L), and Tamaron (100, 150, 200, 250, 300, 400, and 500mg/ L). Lower and/or higher pesticide concentrations were used for some test species. The test flasks were inoculated with 5-day-old cultures of the test organisms, providing an initial algal density of chlorophyll *a* (~26.8–464.5µg/ L). Flasks were incubated for 108 hours at 20–22°C under continuous illumination of 6500–8000 lux. Three replicates of each pesticide concentration were applied. Chlorophyll *a* and protein content were measured to assess toxicity. Pesticide toxicity was expressed as LC50, defined as the minimum pesticide concentration (mg/L) inhibiting algal growth by 50% compared to the control, according to **Walsh *et al.* (1987)**.

Table 1. The organophosphorus pesticide used in this study

Pesticides trade name	Common name	Chemical name	Structural formula
Dimethoate 40%	Sidon,40%EC (Emulsifiable Concentrate)	0,0-dimethyl S-(Z-(methylamino)-2-oxoethyl) phosphorodithioate	$C_5H_{12}NO_3PS_2$
Malathion 57%	Malason	Diethyl (dimethoxyphosphinothioylthio) succinate	$C_{10}H_{19}O_6PS_2$
Tamaron 60%	Methamidophos	O, S-Dimethyl phosphoramidothioate	$C_{17}H_{18}ClF_3N_3O_5PS$

3- Biochemical analysis

The effect of the studied pesticides on chlorophyll *a* and protein constituents of tested microalgae species was studied before and after the toxicity experiments. Algae samples were filtered by Whatman GF/F fiber. Chlorophyll *a* was extracted using 90% acetone, measured according to **APHA (2017)** using PerkinElmer (LS45) fluorescence spectrometer at an excitation wavelength of 430nm and an emission wavelength of 663nm and compared to standard curve as µg L^{-1}. Total protein was analyzed by Biuret method **(David & Hazel, 1993)**.

4- Statistical analysis

Statistical analysis including dose effect analysis, linear regression and one way ANOVA were calculated using XLSTAT *v*2016. Three technical and three biological repetitions were used in each experiment.

RESULTS

The response patterns of the test microalgae species (protein & Chl. *a*) to different organophosphorus pesticides were evaluated using the dose-response curves (Figs. 1, 2). The lethal effective concentration (LC_{50}) is a commonly used metric for assessing the toxicology of substances. Generally, the increase in the LC_{50} values indicates a reduction in pesticide toxicity. The lethal effect of different pesticides varied between species, with no taxon being the most vulnerable to the three pesticides. Malathion is the most toxic pesticide with the lowest LC_{50} of most test species. Tamaron showed a lower toxicity efficiency for most species. The most sensitive species for Tamron was the diatomaceous *G. olivaceum* (LC_{50} of 0.08 mg L^{-1}), while the chlorophytes *Scenedesmus obliquus* and the filamentous cyanobacterium *N. punctiforme* showed comparable LC_{50} levels of 34.48 and 37.5mg L^{-1}, respectively, to the toxicity standard microalga *R. subcapitat* (LC_{50} of 42.0mg L^{-1}). Generally, the response of chlorophyll *a* of different species to the pesticides was like that of the bio-test microalga *R. subcapitata* ($LC_{50;}$ 102.7mg L^{-1} for Dimethoate, 19.58mg L^{-1} for Malathion, and 42mg L^{-1} for Tamron) (Table 2).

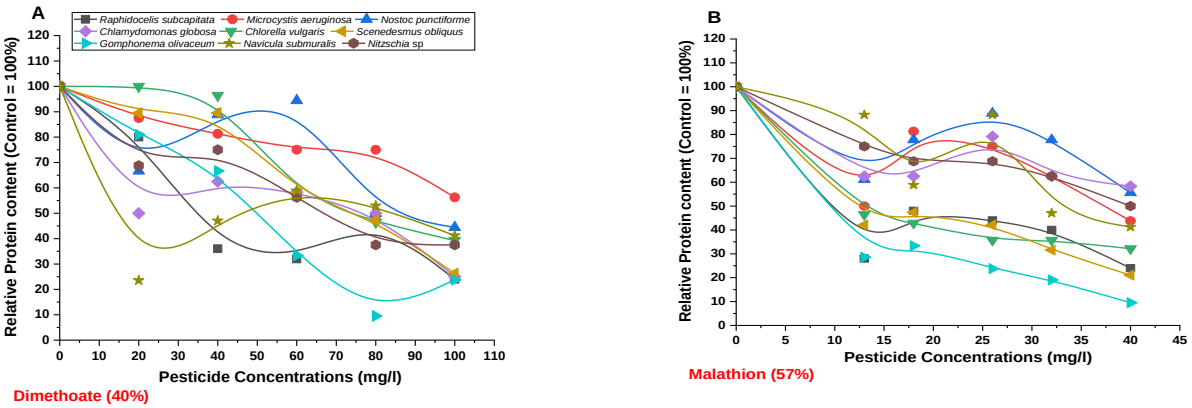

Fig. 1. Dose-response curves of different organophosphorus pesticides; Malathion, Dimethoate and Tamaron on the test microalgae species based on changes in protein content

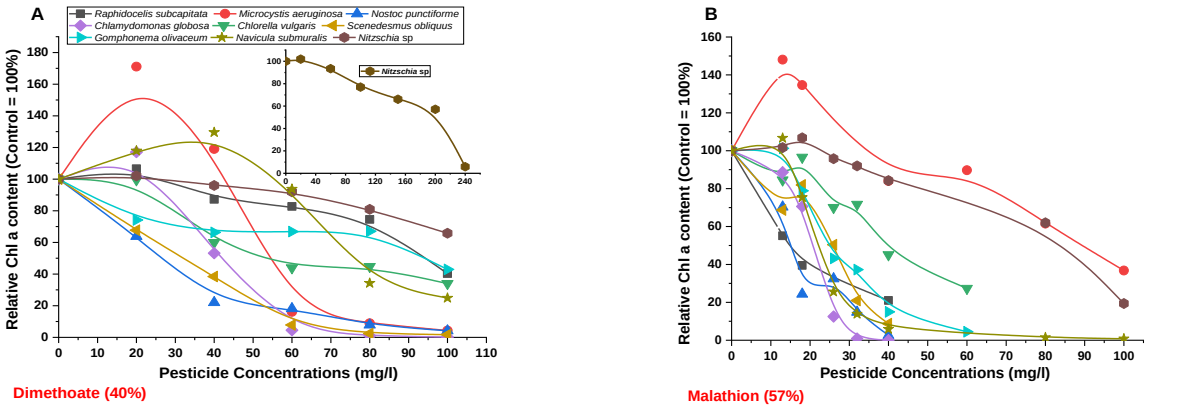

Fig. 2. Dose-response curves of different organophosphorus pesticides; Malathion, Dimethoate and Tamaron on the test microalgae species based on changes in Chl *a* content

Linear regression analysis was employed to explore the strength of the response of protein content and chlorophyll *a* to pesticide concentration. Series of linear regression curves were established for growth experiment under the exposure to different pesticides (Figs. 3a, b; 4a, b and 5a, b).

Impact of Dimethoate exposure on protein & chlorophyll *a* content

When freshwater microalgae were exposed to increasing concentrations of Dimethoate, their growth was significantly inhibited. Dimethoate treatments at varying dosages significantly reduced Chl *a* level in cells. The average 96-h LC_{50} for different microalgae after the exposure to Dimethoate dosages based on protein and Chl *a* were 98.78 and 77.07mg L^{-1}, respectively. The 96-h LC_{50} for protein varied between 40.32mg L^{-1} for *Navicula submuralis* and 236.74mg L^{-1} for *Microcystis aeruginosa*. LC_{50} for Chl *a* were 25.28 & 28.4mg L^{-1} for *N. punctiforme* & *S. obliquus*, respectively, and 169mg L^{-1} for *Nitzschia* sp. The results showed that *Nitzschia* sp. was more tolerant to Dimethoate showing higher LC_{50} for protein (165.34 mg L^{-1}) and Chl. *a* (169 mg L^{-1}). Protein is less affected than Chl *a* and the most sensitive taxon had the lowest LC_{50}. The average LC_{50} for the tested microalgae were 98.78 and 77.04mg L^{-1} for protein and Chl-*a*, respectively. On the other side, the average R^2 of the linear regression analysis showed that protein was less associated (R^2 =0.731) with Dimethoate exposure than Chl *a* (R^2 =0.809). The regression coefficient of both protein and chl *a* of different species against Dimethoate concentrations were non-significantly varied (*P*= 0.333) revealing the similar pattern of response of both protein and chlorophyll *a* for different species. *G. olivaceum* (R^2= 0.895 for protein and 0.796 for chlorophyll, respectively) had the exact same pattern of *R. subcapitata* (R^2= 0.726 for protein and 0.836 for chlorophyll, respectively) as their protein contents are more impacted by Dimethoate than Chl. *a*. While Chl. *a* contents of *Scenedesmus obliquus (*LC_{50}= 28.4 mg L^{-1} & $R^{2=}$ 0.898), *Microcystis aeruginosa* (LC_{50}= 52.2 mg L^{-1} & $R^{2=}$ 0.657), and *Nostoc punctiforme* (LC_{50}= 25.28mg L^{-1}& $R^{2=}$ 0.842) were more sensitive to Dimethoate (Table 2 & Fig. 2a, b).

Impact of Malathion exposure on protein & chlorophyll *a* content of nine freshwater microalgae

The impact of pesticide Malathion on Chl-*a* and total protein of nine freshwater algal species was examined. Malathion's LC_{50} values were 10.02, 16.84, 11.76, 11.32, 36.72, and 41.96mg L^{-1} for protein of *R. subcapitata, C. vulgaris, S. obliquus, G. olivaceum, N. submuralis and Microcystis aeruginosa*, respectively, (Table 2 and Fig. 4a &b). The results indicated that protein content was more significantly affected than chlorophyll a (Chl. a) in the tested algal species. The average LC_{50} for the tested microalgae were 38.64 and 40.08mg L^{-1} for protein and Chl-*a*, respectively. On the other side, the average R^2 of the linear regression analysis showed that the association of

protein and Chl *a* response of the tested microalgae to Malathion exposure were R^2 =0.647 and R^2 =0.788, respectively. The regression coefficient of both protein and Chl *a* of different species against Dimethoate concentrations were non-significantly varied (*P*= 0.479) revealing the similar pattern of response of both protein and chlorophyll *a* for different species. In contrast with the remainder of the algal species, there was a drastic reduction in Chl. *a* content of *C. globosa, Nitzschia* sp., and *N. punctiforme* (r =-0.938, -0.786, and -0.94, respectively).

Impact of Tamaron exposure on protein & chlorophyll *a* content of nine freshwater microalgae

Vast differences were detected in LC_{50}, r, and R^2 values between different species of microalgae (Table 2 & Fig. 5a, b). Protein contents were much lower affected by Tamaron than Chl *a* in these algal species and the most sensitive taxon had the lowest LC_{50}. Protein contents showed much higher average LC_{50} for the tested microalgae (372.3 mg L^{-1}) compared with Chl *a* response (192.5 mg L^{-1}) by the exposure to Tamaron. When the average R^2 of the linear regression analysis showed that the association of protein and Chl *a* of the tested microalgae to Tamaron exposure were R^2 =0.789 and R^2 =0.611, respectively, their regression coefficients were high-significantly varied (*P*= 0.015) revealing the great different pattern of response of both protein and chlorophyll *a* for different species. LC_{50} value of protein of *C. globosa* (2020.8mg L^{-1}) was 4-fold greater than that of Chl *a* (509.04mg L^{-1}). Standardized coefficient (r) derived from total protein content of *R. subcapitata, S. obliquus, G.olivaceum N. submuralis, N. punctiforme* were -0.816, -0.808, -0.828, -0.886, and -0.911 , while from Chl *a* were -0.803, -0.659, -0.495, -0.656, and -0.71. This indicated that these species protein was more tolerant to Tamaron. Unlike to the previous observations, LC_{50} values for Chl *a* of *C. vulgaris, Nitzschia* sp., and *M. aeruginosa* were greater than that of protein of these algal species.

Table 2. LC_{50}, r, and R^2 of the test microalgae species exposed to different organophosphorus pesticides; Dimethoate, Malathion and Tamaron

Organism	Dimethoate						Malathion						Tamaron					
	LC_{50} mg L^{-1}		Standardized coefficient(r)		Regression (R^2)		LC_{50} mg L^{-1}		Standardized coefficient(r)		Regression (R^2)		LC_{50} mg L^{-1}		Standardized coefficient (r)		Regression (R^2)	
	protein	Chl. *a*	protein	Chl. *a*	protein	Chl. *a*	protein	Chl. *a*	protein	Chl. *a*	protein	Chl. *a*	protein	Chl. *a*	protein	Chl. *a*	protein	Chl. *a*
Raphidocelis subcapitata	43.58	102.7	-0.852	-0.914	0.726	0.836	10.02	19.58	-0.751	-0.902	0.564	0.813	49.84	42	-0.816	-0.803	0.666	0.645
Chlorella vulgaris	80.08	63.16	-0.94	-0.936	0.883	0.876	16.84	59.26	-0.855	-0.77	0.731	0.594	190.96	405.62	-0.926	-0.904	0.857	0.817
Chlamydomonas globosa	48.66	40.54	-0.827	-0.909	0.685	0.827	54.02	19.38	-0.797	-0.938	0.499	0.88	2020.8	509.04	-0.947	-0.965	0.896	0.932
Scenedesmus obliquus	80.88	28.4	-0.969	-0.947	0.94	0.898	11.76	22.28	-0.9	-0.955	0.809	0.912	63.02	34.48	-0.808	-0.659	0.653	0.434
Gomphonema olivaceum	43.46	132.32	-0.946	-0.892	0.895	0.796	11.32	26.36	-0.872	-0.939	0.761	0.881	76.8	0.08	-0.828	-0.495	0.686	0.245
Navicula submuralis	40.32	79.96	-0.405	-0.81	0.164	0.657	36.72	83.96	-0.825	0.955	0.68	0.912	207.62	84.26	-0.886	-0.656	0.785	0.43
***Nitzschia* sp.**	165.34	169	-0.916	-0.942	0.84	0.888	66.56	22.8	-0.966	-0.786	0.933	0.618	188.04	256.14	-0.923	-0.91	0.851	0.829
Microcystis aeruginosa	236.74	52.02	-0.96	-0.81	0.922	0.657	41.96	98.42	-0.707	-0.77	0.499	0.595	193.78	363.58	-0.938	-0.813	0.881	0.661
Nostoc punctiforme	149.98	25.28	-0.726	-0.918	0.527	0.842	98.54	15.94	-0.587	-0.94	0.345	0.883	360.18	37.5	-0.911	-0.71	0.83	0.505
Average	98.782	77.042	-0.838	-0.898	0.731	0.809	38.638	40.887	-0.807	-0.672	0.647	0.788	372.338	192.522	-0.887	-0.768	0.789	0.611
ANOVA	P = 0.346				P = 0.333		P = 0.922				P = 0.497		P = 0.003				P = 0.015	

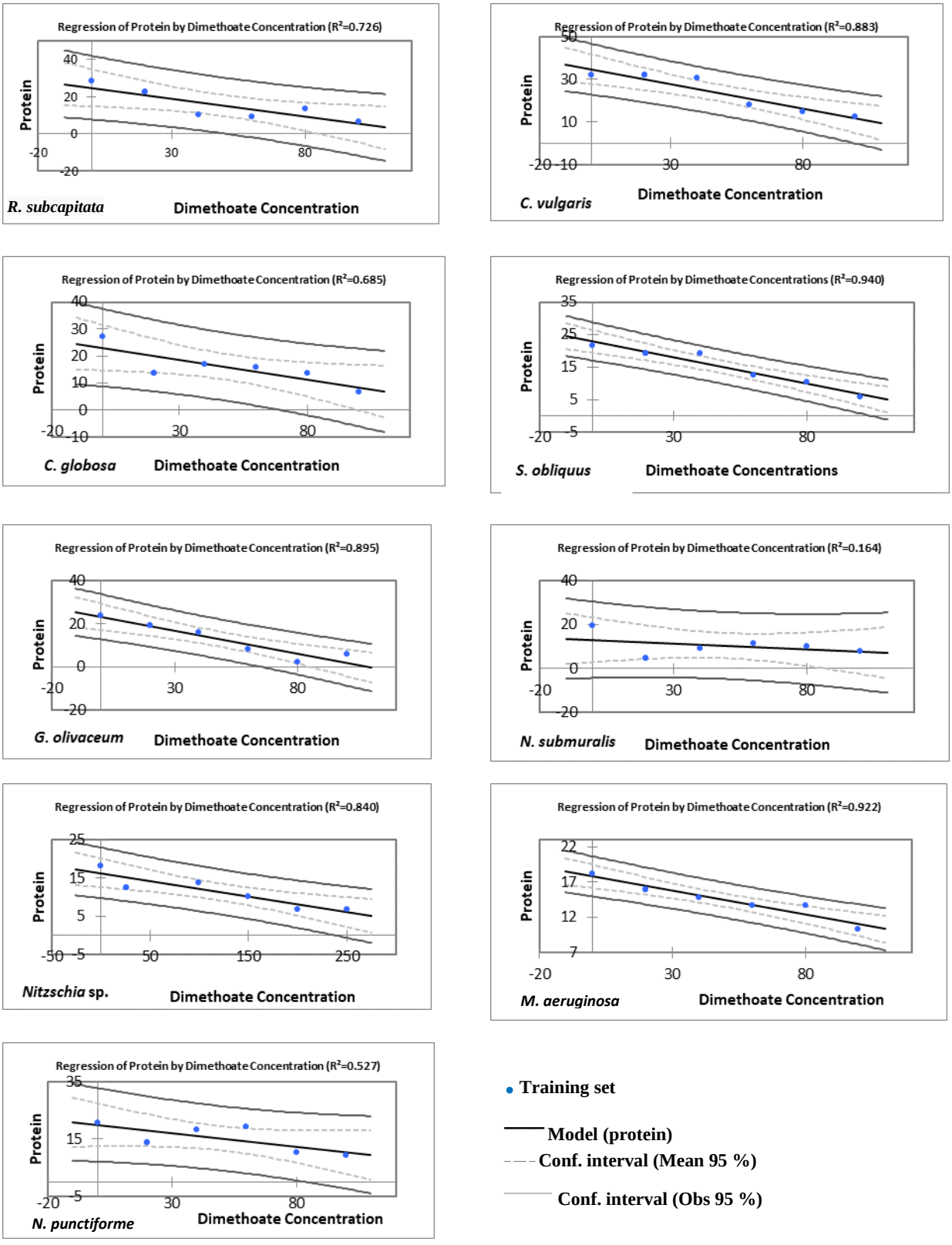

Fig. 3a. The regression (R^2) of protein by Dimethoate concentrations for 9 micro algal species

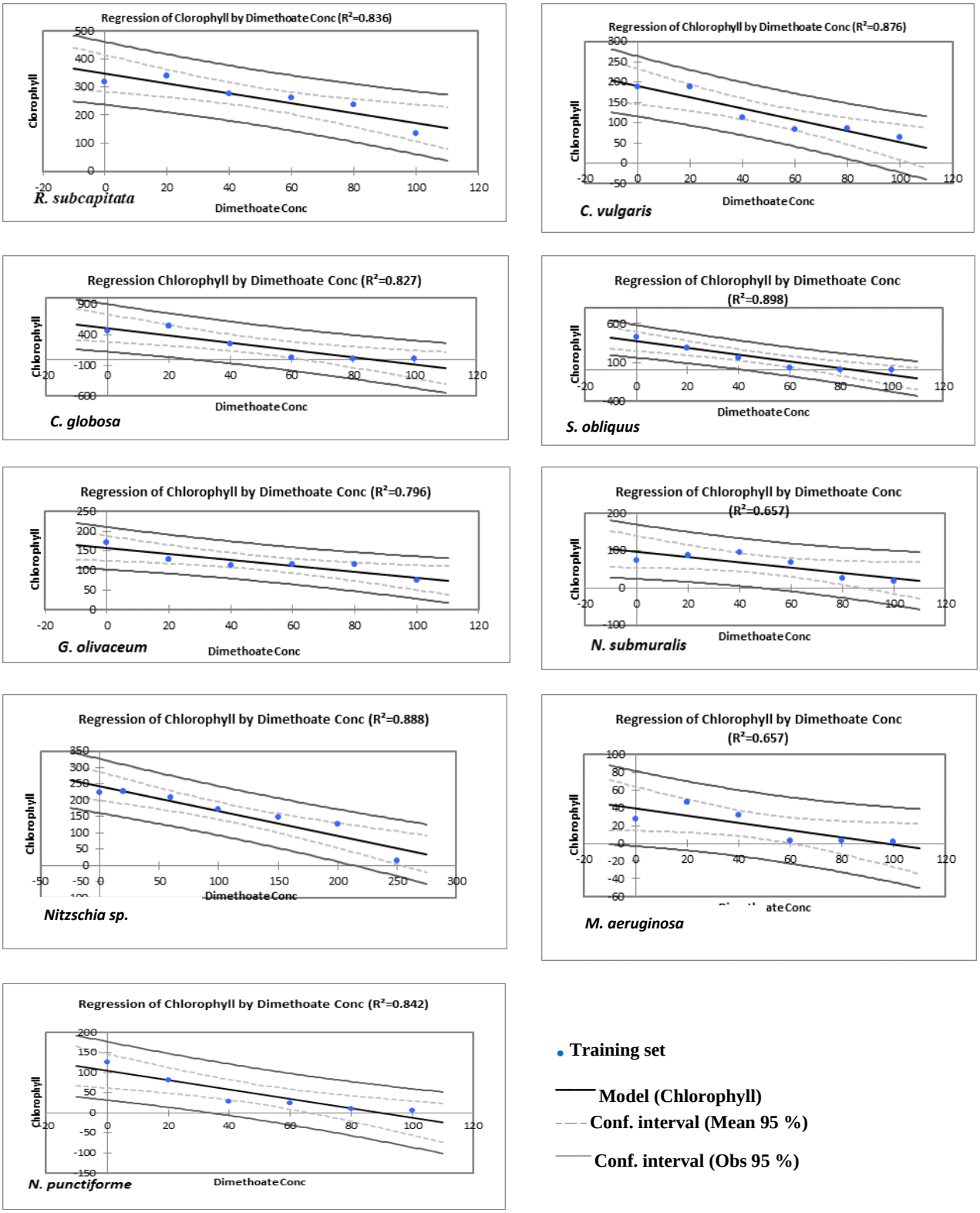

Fig. 3b. The regression (R^2) of chlorophyll *a* by Dimethoate concentrations for 9 micro algal species

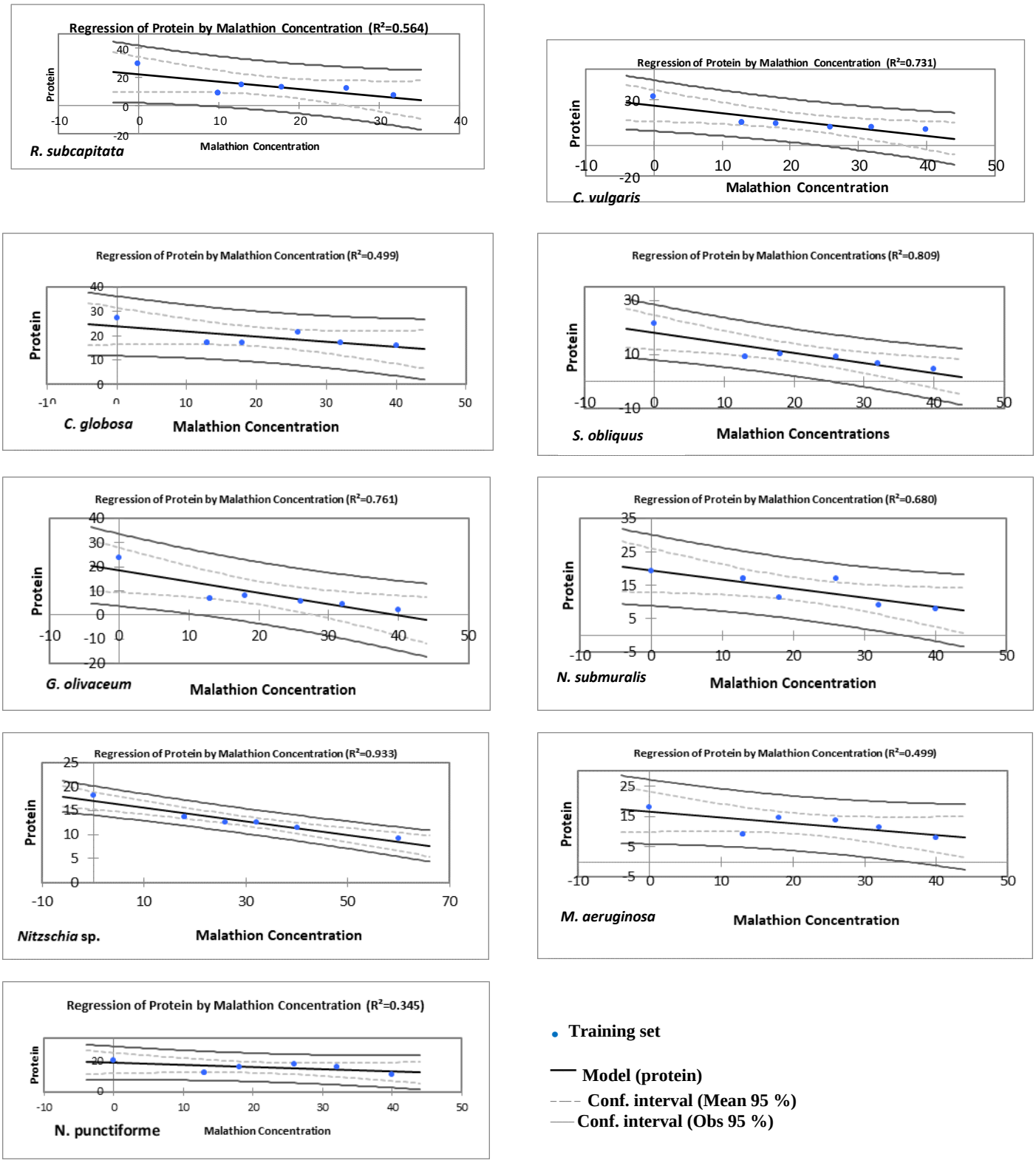

Fig. 4a. The regression (R^2) of protein by Malathion concentrations for 9 micro algal species

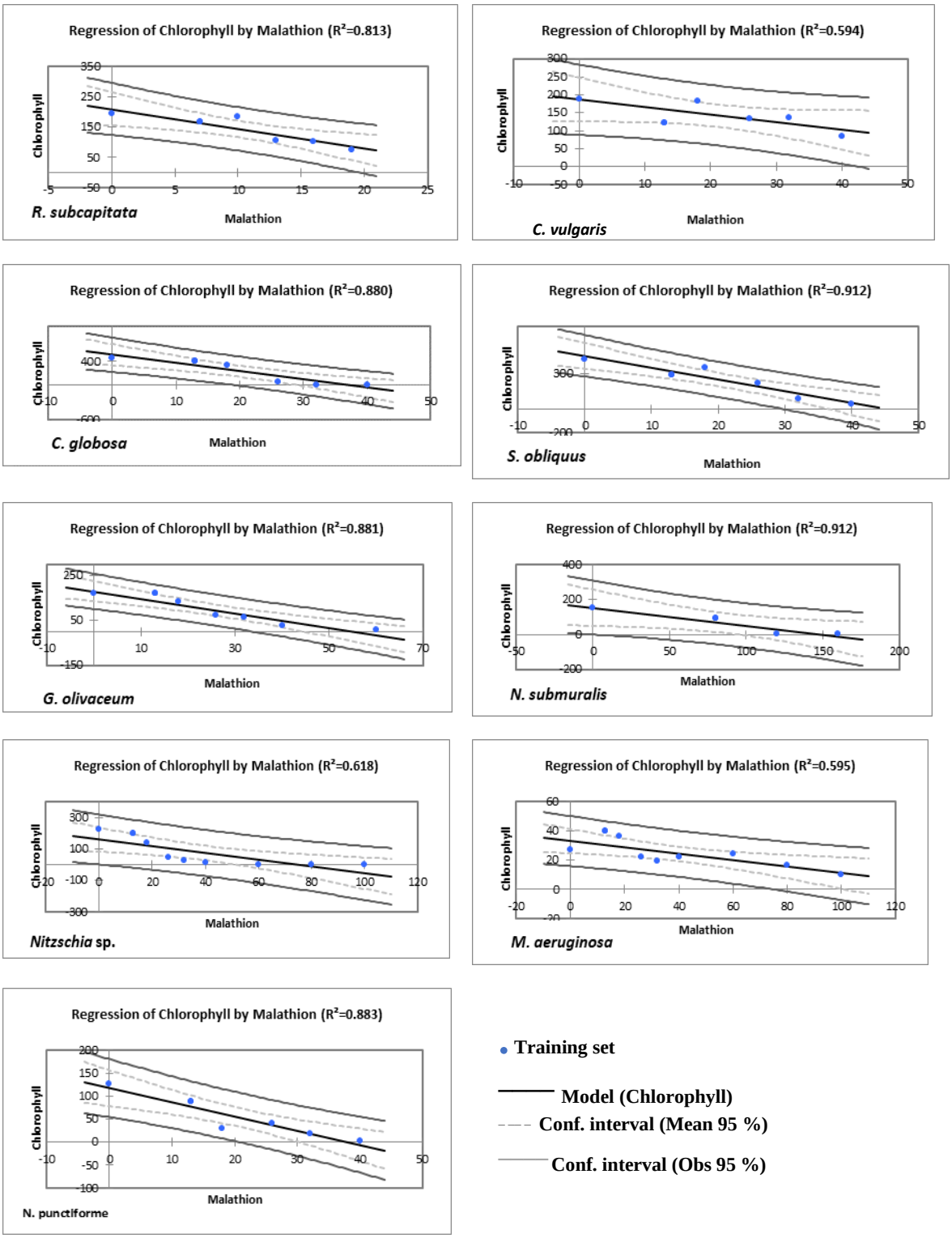

Fig. 4b. The regression (R^2) of Chl. *a* by Malathion concentrations for 9 micro algal species

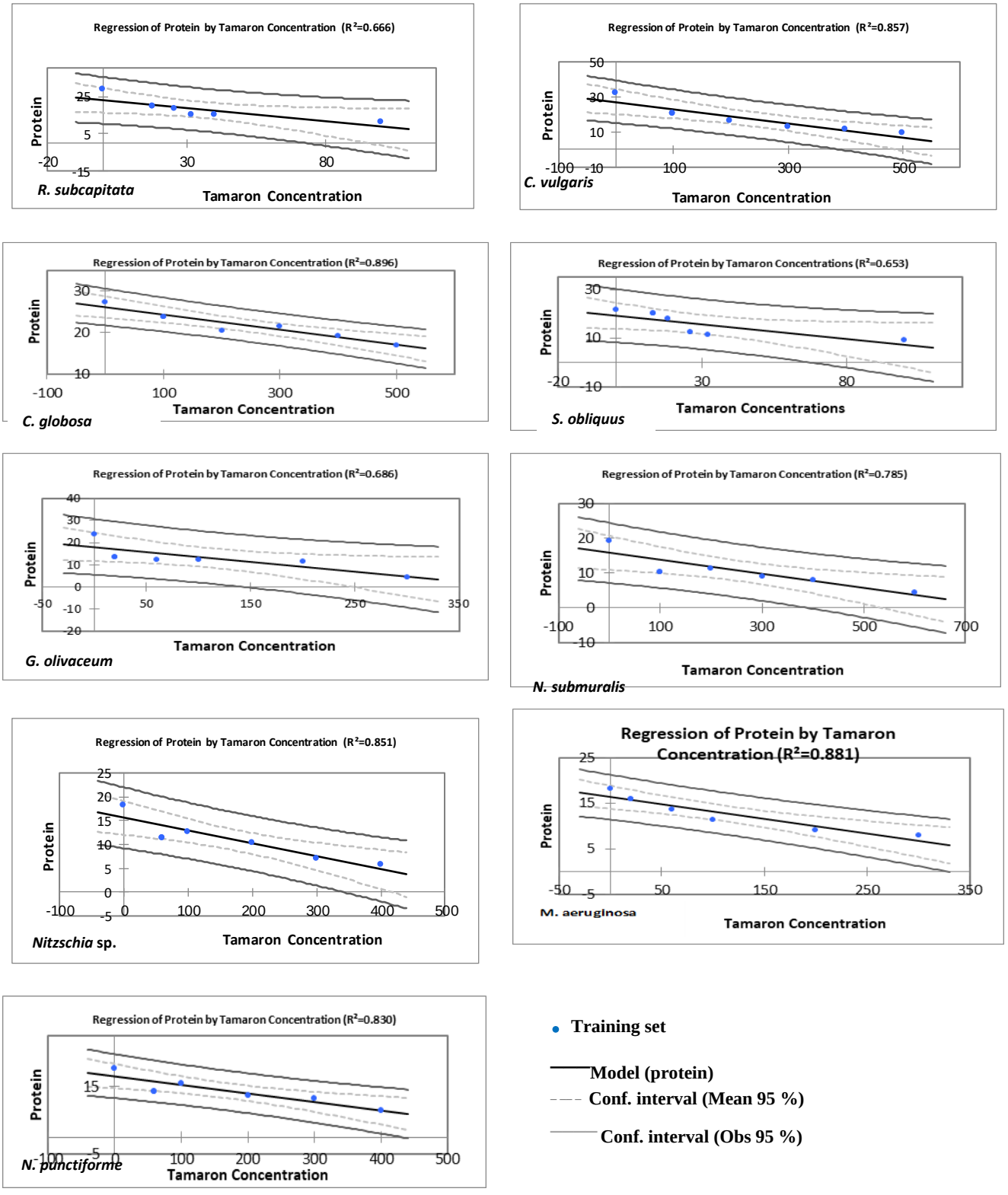

Fig. 5a. The regression (R^2) of protein by Tamaron concentrations for 9 micro algal species

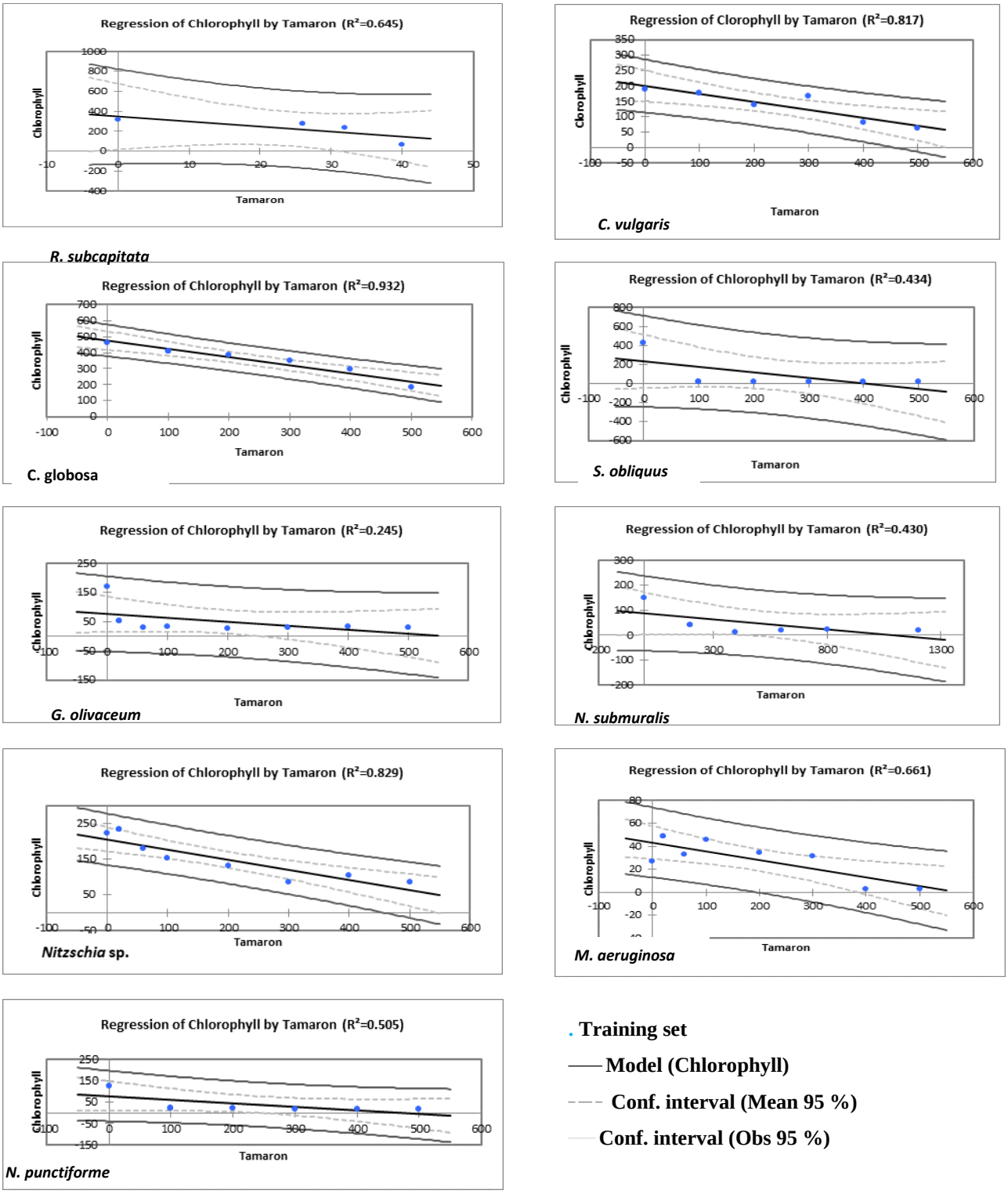

Fig. 5b. The regression (R^2) of Chl *a* by Tamaron concentrations for 9 micro algal species

DISCUSSION

The lethal effective concentration (LC_{50}) is a commonly used metric for assessing the toxicology of substances **(Huang *et al*., 2012a, 2012b; Abo Arab *et al*., 2022)**. The increasing use of pesticides in agriculture to increase crop yield harms aquatic ecosystems, damaging environmental integrity and human health. Pesticides contaminate water bodies through runoff, chemical spills, and effluent, harming algae, which are important primary producers in aquatic ecosystems **(Narayanan *et al*., 2024)**. Phytotoxicity tests, which inhibit microalgae development, are often used to assess a compound's toxicity. Cell density is a commonly used toxicity assessment tool (**Xiong *et al*., 2016)**. Certain species are impacted because they metabolize these chemical pollutants and store them in their bodies, magnifying their effects on the ecosystem as a whole **(Dhanker *et al*., 2023)**. In this paper, we examined if protein content alone can be used as a direct indicator of pesticide toxicity on different species of microalgae as chlorophyll *a* content or not. Thus, the effects of pesticides Dimethoate, Malathion, and Tamaron on Chl *a* and the total protein of nine freshwater phytoplankton were studied.

As indicated in the results section, the toxicity of examined microalgae differed greatly due to pesticides' various chemical structures. The 96-h LC_{50} for protein varied around 40.32 mg L^{-1} (for *N. submuralis*, r=-0.405*)* and 236.74 mg L^{-1} (for *M. aeruginosa*, r= -0.96) in case of Dimethoate. Total protein contents of all tested diatom species were more resistant for pesticide Dimethoate than their Chl *a* (r=-0.946, -0.405, and -0.916 for *G. olivaceum, N. submuralis, Nitzschia* sp., respectively). Diatoms against pesticide Dimethoate had the same pattern as standard green algae *R. subcapitata* ($LC_{50=}$ 43.58 & 102.7 mg L^{-1} for protein & Chl *a*, respectively). **Jampani and Kumari (1988)** stated that Dimethoate reduced growth of *Scenedesmus incrassatulus* at doses above 0.075 and 0.5 mg L^{-1}. This pesticide significantly reduced the quantity of algal proteins. **Mohapatra and Mohanty (1992)** found that the 10-day LC_{50} for *Chlorella vulgaris* and *Anabaena doliolum* treated by Dimethoate were 51 and 28.5mg L^{-1}, respectively. On the other hand, the protein of the rest of tested algae was more tolerant to Dimethoate than Chl *a*, especially that of blue greens *M. aeruginosa* and *N.punctiforme*. This contrasts the findings of **Wu *et al*., (2016)**, who found that changes in cell density, chlorophyll *a*, and protein content are constant after] *M. aeruginosa* exposure to glyphosate (herbicide). Diatoms showed lower sensitivity to pesticides compared to green algae. These results support the conclusions of **Tomlin's (2009)**, who indicated that green algae are more sensitive than diatoms. Freshwater biofilms are mostly produced by the diatoms *Nitzschia palea* and *Nitzschia pelliculosa*, which create extracellular polymeric substances. Diatoms' higher tolerance may be due to the presence of fucoxanthin in their plastids, which protects them against photo-oxidation caused by pesticides **(Sutherland, 2001; Larras *et al*., 2013)**. According to the study of **Mavrogenis *et al*., 2023**, the mode of action of Dimethoate in the aquatic environment has been unclear till now, and it lowers

the rate of photosynthetic oxygen evolution. **Mohapatra *et al*. (1997)**, **Leboulanger *et al*. (2009)** and **Mohapatra and Schiewer's (2000)** investigated the effects of Dimethoate on blue green algae *Synechocystis* sp. photosynthesis and respiration. They found that the insecticide increased respiratory oxygen consumption at all tested dosages (10-300 μM), but significantly reduced photosynthesis at concentrations ≥50 μM. In addition, pesticide altered the algal fluorescence behavior and pigment concentration due to membrane interactions.

Except for three species (green algae *C. globosa*, diatom *Nitzschia* sp., and blue green algae *N. punctiforme*), chlorophyll *a* in all studied microalgae was more resistant to pesticide Malathion than protein content. Chlorophyll *a* is an essential component of the photosynthetic machinery of microalgae. It is essential for light absorption and energy transfer **(Jian-Ren Shen, 2021; Tomo & Allakhverdiev 2021)**. The result of this point is similar to the findings of **Selvakumar *et al.* (2000)**, who found that *Anabaena* sp. may tolerate various doses of butachlor pesticide without significantly reducing chlorophyll *a* content. Microalgae must have a high chlorophyll *a* content to survive and flourish in many different kinds of environments **(Juneja *et al*., 2013; Da Silva & Lombardi, 2020)**.

At high doses, Tamaron had a negative effect on tested algae. Protein content was more resistant to this pesticide (R^2= 0.666, 0.896, 0.653, 0.686, 0.785, and 0.83 for *R. subcapitata, C. globosa, S. obliquus, G. olivaceum, N. submuralis,* and *N. punctiforme*) **Marchingo and Cantre (2022)** stated that in algal cells, protein synthesis and metabolism take place via complicated, interrelated pathways. Therefore, pesticides may be more effective at disrupting specific protein production pathways, resulting in alterations in overall protein composition. However, algae may have a few redundant steps in these mechanisms, allowing them to maintain protein levels to some degree.

It is important to note that the relative sensitivity of chlorophyll *a* and protein content varies based on the pesticide, method of action, and microalgal species under study **(Be´rard *et al*., 2004; Chin *et al*., 2019; Xu *et al*., 2024)**. Changes in total protein composition, while critical for cellular metabolism and growth, may have a less direct and severe influence on algal cell viability than disturbances in the photosynthetic system. **Gerin *et al*. (2016)** and **Chambonniere *et al*. (2022)** emphasized that although changes in total protein levels may not immediately threaten cell survival, they are essential for metabolic processes. For instance, *Chlorella vulgaris* showed a reduction in protein concentration during nutrition limitation while keeping some metabolic activity.
Chlorophyll *a* and protein content can be used to evaluate the way pesticides affect microalgae, providing unique insights into their physiological reactions. Protein concentration indicates more extensive metabolic alterations, whereas chlorophyll *a* is a direct measure of photosynthetic activity. To comprehend the ecological effects of

pesticides on aquatic environments, both metrics are essential **(Chen *et al*., 2016; Gao *et al*., 2016; Moreira *et al*., 2020)**.

CONCLUSION

Protein content in microalgae can provide valuable insights into the overall health of the cells. Meanwhile, **chlorophyll *a* (Chl. *a*)** concentration is an important indicator when assessing the impacts of pesticides, as it directly reflects photosynthetic activity. For a comprehensive evaluation of pesticide effects, it is essential to consider both **protein concentrations** and **Chl. a content** in microalgae exposed to pesticides.

Contrary to most previous studies, the present research suggests that **pesticide toxicity** in microalgae can be directly assessed through **protein content alone**, particularly when the differences in **LC50** values for the tested microalgae were not significant for both **Dimethoate** and **Malathion**, but were highly significant for **Tamaron**. This finding highlights the potential of protein content as a more reliable indicator of pesticide toxicity in certain cases.

Future research comparing the responses of **protein** and **chlorophyll** content in microalgae exposed to a wider range of pesticides, especially **organochlorine pesticides**, is essential. Such studies would help address existing knowledge gaps and improve our understanding of the complex interactions that drive these ecological processes.

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