Biomass Conversion and Biorefinery
https://doi.org/10.1007/s13399-023-03949-9

ORIGINAL ARTICLE

Potential impacts of *Ascophyllum nodosum*, *Arthrospira platensis* extracts and calcium phosphite as therapeutic nutrients for enhancing immune response in pepper plant against *Fusarium* wilt disease

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Received: 4 January 2023 / Revised: 6 February 2023 / Accepted: 9 February 2023

Abstract
The search for active inducers against diseases in the formula of therapeutic nutrients has become a necessity for many researchers. The study's chief purpose was to make agronomic farming simpler by applying newly created therapeutic nutrients. The novelty of this research is the applied of algal extracts in adding to minerals as therapeutic nutrion. Calcium (Maxifos Ca), *Ascophyllum nodosum* (Greencal), and *Arthrospira platensis* (*A. platensis*), were tested for induction pepper plant resistance against *Fusarium* wilt. The disease index (DI), morphological growth, photosynthetic pigments, free proline, total phenol, hydrogen peroxide (H_2O_2), malondialdehyde (MDA), and antioxidant enzymes as reactions to the induction of protection in challenged tested plants were measured. Results revealed that the use of entirely different treatments significantly minimized the danger of *Fusarium* wilt. Treatment of infected plants with Maxifos Ca was the best treatment, as it reduced the DI to 25% and thus reduced symptoms and improved the percentage of plant protection from the disease by 69.6%. Surprisingly, it was widely assumed that Greencal was the greatest treatment for restoring vegetative growth, followed by Maxifos Ca and an algal extract, *A. platensis*. The application of Greencal, followed by Maxifos Ca, and then *A. platensis* significantly increased the expression of all metabolic resistance indices (phenols, polyphenol oxidase, and peroxidase). The best treatments for reducing the signs of stress represented in (MDA and H_2O_2) were Maxifos Ca and then Greencal. According to the findings the use of Maxifos Ca, Greencal, and *A. platensis* as alternate therapeutic nutrients of eco-destructive chemically synthesized fungicides appears to be a significant methodology for reducing the harmful effects of *Fusarium* wilt on pepper plants.

Keywords *A. nodosum* · *A. nodosumm, Fusarium* · Plant immunity · Wilt disease

1 Introduction

The plant has the ability to attack risks and challenges, and its ability to resist these risks depends entirely on its nutritional status [1]. The more plant diseases, the greater the consumption of pesticides to eliminate pathogens and protect the agricultural economy [2–4]. *Capsicum annuum* L. is a vital crop cultivated widely all over the world [5–7]. *Fusarium* fungus is considered one of the most dangerous pathogens of the pepper plant [8]. It is present in all types of agricultural soils, whether organic or conventional [9]. Fungi are considered one of the most dangerous pathogens of the pepper plant, and *t* is present in all types of agricultural soils, whether organic or inorganic. Despite the use of chemically synthesized fungicides being one of the most effective means of controlling fungal plant diseases, it is considered very harmful to the environment and climate [10, 11]. Indiscriminate and excessive use of chemical pesticides adversely affects soil vitality, plant health, and human health [9]. Natural inducers can stimulate the plant to defend against pathogens and increase productivity without affecting the

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Published online: 28 February 2023

vitality and fertility of the soil and at the same time therapeutic nutrients [12, 13]. Therapeutic nutrition is a diet that determines giving the plant nutrients and fertilizers that activate and stimulate physiological processes and help improve the plant's ability to face stress and risks and reduce some diseases or side effects associated with those diseases [2, 14–16]. It is scientifically recognized that algae are one of the most powerful growth-stimulating organisms that push the plant to produce effective substances capable of raising the efficiency of physiological immunity from the formation of hormones, proteins, and phenolic substances and activating the work of antioxidant enzymes [16–18]. Macroalgae extract produces substances that work to block the progress of the pathogen or limit its progression and endurance of stress conditions and reduction of oxidative blast in cells [19, 20]. Algae produce compounds that inhibit the activity of plant pathogens, such as phenolics, and their oxidized products, which are considered more toxic to pathogens [21, 22], it releases phenols that are toxic to phyto pathogens [23]. Thus induced resistance is that resistance that is activated by biological or abiotic factors, which leads to the presence of some natural and chemical obstacles in the activated plant, which is a change in the plant's physiology resulting from the acquired traits [24, 25]. Resistance inducers affect the host plant at levels of morphology, anatomy, or the creation of definite chemicals that restrict the phytopathogens or minimize the disease severity [26, 27]. Marine algae are considered an actual bio-fungicide for phytopathogens through algal bioactive metabolites such as oleic acid, fatty acid esters, palmityl, and myristic alcohol [28, 29]. *A. platensis* extract contains phenolics that resulted in their antifungal activity [30, 31]. Calcium is an important mineral that encourages plant expansion through a variety of physiological routes [32], and plant tissues as cell wall breadth, and rebuilding [33]. The most important characteristic of the use of *A. nodosum* extract as therapeutic nutrients in plants is that it contains a substance: alginic acid, a natural chelating substance that chelates Fe, Zn, Mn, Mg, and Ca and activates the formation of polysaccharides and activates the formation of natural growth regulators, polyamine, and natural antibiotics within the plant [34]. Recently, phosphites and phosphonites have taken over the market as phytopathogens fungicides, providing a powerful preventive impact by stimulating defense mechanisms [35]. Therefore, the major goal of this study was to explore the activities of Greencal, Maxifos ca, and *A. platensis* to reduce the destructive effect of *F. oxysporum* on pepper as well as enhance plant growth by improving physiological immune responses.

2 Materials and methods

2.1 Source of pathogen *F. oxysporum*

The pathogen was received from Regional Center for Mycology et al.-Azhar University (RCMB) and confirmed according to Hibar et al. [36].

2.2 Source of inducers

Maxifos Ca® (calcium phosphite) and Greencal® (Ascophyllum nodosum extract) as a bio-stimulant obtained by AL-SALAM International for Development and Agriculture Investment, Egypt from MAFA-VEGETAL ECOBIOLOGY-Spain. *Arthrospira platensis* HSSASE5 KT277788 obtained from the botany and microbiology department, science faculty, Cairo University.

2.3 Pot experimental

The experiment was conducted at the experimental farm of ALSALAM International for Development and Agriculture Investment, Egypt.

Three-week-old pepper seedlings were cultivated in 40 × 40 cm pots, with every treatment having six seedlings. At a temperature of 22 °C and a relative humidity of 80%, the pots contained 7 kg of 1:3 sandy clay. The pathogen. *F. oxysporum* (10^7 spore / mL) was putted into pots. Maxifos Ca®, Greencal® and *A. platensis* (3 cm/L) spraying on the pepper leaves three times. Three replicates of each treatment were arranged in a completely randomized: T1-control healthy, T2-control infected, T3-healthy and Maxifos Ca®, T4-health and Greencal®, T5-healthy and *A. platensis*, T6-infected pepper and Maxifos Ca®, T7-infected pepper, and Greencal® and T8-infected pepper and *A. platensis*).

2.4 Disease index

DI and protection were evaluated according to Attia et al. [37], with minor variations. The percent disease index (PDI) was firm using this equation: PDI = (1n 1 + 2n2 + 3n3 + 4n4)100/4nt, where n1–n4 represents the number of plants in each class and nt symbolizes the total number of plants studied. And the following equation was used to calculate % Protection.

% Protection = A − B/A 100%, where *A* is the PDI in infected control plants and *B* is the PDI in infected plants treated with different treatments.

2.5 Metabolic indicators for pepper resistance

Of The determination of photosynthetic pigments was accomplished by the technique of Abdelaziz et al. [38] with minor alternations, the mount of chlorophyll a (Chl a), chlorophyll b (Chl b) as well as carotenoids in fresh leaves. pigments were extracted by dissolving (0.5 g fresh leaves) in 50 mL of 80% acetone, then filtered with filter paper Whatman no 1 then the obtained color was assayed spectrophotometrically at 665, 649, and 470 nm. These equations were used to caculate the pigments; mg chlorophyll (a)/g fresh leaves = 11.63(A665) − 2.39(A649), mg chlorophyll (b)/g fresh leaves = 20.11(A649) − 5.18(A665), mg chlorophyll (a + b)/g fresh leaves = 6.45 (A665) + 17.72(A649), and Carotenoids = $1000 \times O.D_{470} - 1.82\ C_a$—$85.02\ C_b/198$ = mg/g fresh weight. "A" means the optical density.

A procedure by Umbreit et al. [39] was used for testing the total soluble sugars in the pepper-dried tissues, where 0.5 g dried plant shoots was mixed with 5 mL of 30% trichloroacetic acid and 2.5 mL of 2% phenol then filtered, then 1 mL of the mixture filtrate was treated with 2 mL of anthrone reagent (2 g anthrone/L of 95% H_2SO_4) then readied at 620 nm.

Soluble proteins were determined by the method Lowry et al. [40]. One gram of the dried tissues was extracted by mixing with 5 mL of 2% phenol water and 10 mL of distilled water was added; the solution was shaken for 12 h, filtered, and recompleted volume to 50 mL with DW; then One mL of this filtrate was combined with 5 mL of solution (50 mL of 2% Na_2CO_3 prepared in 0.1N NaOH and 1 mL of 0.5% $CuSO_4$ prepared in 1% potassium sodium tartrate) and 0.5 mL of Folin's reagent (1:3 v/v). After 0.5 h, optical density was determined at 750 nm.

Free proline was estimated by the method of Bates et al. [41], and 0.5 g dried shoots was extracted by 10 mL of sulfosalicylic acid (3%), then 2 mL of the extract was mixed with 2 mL of ninhydrin acid and 2 mL of glacial acetic acid for an hour under boiling conditions, then stop the reaction by ice. Finally, 4 mL of toluene was added to the mixture and assayed at 520 nm.

Procedures by Dai et al. [42] were applied to measure the plant phenolics. One gram of dried pepper tissues was extracted in 10 mL of ethanol 80% for 1 day. Then re-extracted using 10 mL of ethanol 80%. The filtrate was then refilled to 50 mL with 80% ethanol, and then 0.5 mL of the filtrate was mixed well with 0.5 mL of Folin's reagent with shaken for 3 min, then 3 mL of DW and 1 mL of saturated sodium carbonate solution was added and thoroughly mixed then detected at 725 nm. The procedure of [43] was used to assay the MDA content in fresh plant leaves. Fresh pepper leaves also were established for hydrogen peroxide H_2O_2 content [44]. Embraced method of Srivastava [45] was used to determine POD. The activity of PPO was stately by the method of Hashem et al. [8].

2.6 Statistical investigates

A one-way analysis of variance (ANOVA) was applied to the resulting data. LSD by CoStat (CoHort, Monterey, CA, USA) was applied to demonstrate statistically relevant variances at $p < 0.05$ [46].

3 Results

3.1 Disease assessment

The data in Table 1 and Fig. 1 are shown that *F. oxysporum* infection produced a great percent disease index (PDI) of 82.5%. Reducing the seriousness of the disease is the first mark of the efficacy of the tested Maxifos Ca, Greencal, and *A. platensis* extract in stimulating plant resistance. The data exhibited that treatment with the Maxifos Ca and Greencal recorded high protection by 69.6% and 63.63% and the lowest PDI to 25% and 30% and came next *A. platensis* extract PDI by 37.2%.

3.2 Growth markers

The presented results in (Fig. 2) showed that *Fusarium* wilt damaged all pepper growth traits in contrast with healthy control. Regarding the effect of Greencal, Maxifos Ca, and *A. platensis* extract, it was detected that healthy plants treated with Greencal and Maxifos Ca respectively showed highly promising recovery. When it came to the effects of the treatments on the infected plants, it was noticed that GreenCal had the greatest efficacy for increasing plant growth (shoot and root lengths), followed by Maxifos Ca, and then algal extract *A. platensis*. On the other hand, Maxifos Ca induced the highest number of leaves, followed by Greencal and *A. platensis*.

3.3 Photosynthetic pigments

The data shown in Fig. 3 proved that *Fusarium* wilt resulted in a major shortage of chlorophyll pigments (a and b) as well as carotenoid content by 51.95%, 16.13%, and 60.62%. The results are obtainable for the recovery of photosynthetic pigments due to employing all treatments. On the other hand,

it was established that using of Greencal was the greatest inducer to augment plants Chl a, b, and carotenoids of both healthy and *F. oxysporum*–infected plants. For more, it was found that all of the tested inducers caused an improvement in photosynthetic pigments.

3.4 Free proline and phenol content

The results in Fig. 4 indicated that the *Fusarium* wilt-infected plants showed an increase in the free proline and phenol contents by 5.88% and 22.5%. On the other hand, the application of tested elicitors Maxifos Ca, Greencal and *A. platensis* extract enhanced the resistance of the plant by increasing free proline and phenol contents. Concerning the effect of tested elicitors on both (healthy and infected), it was established that all tested elicitors trigger an increase of free proline and phenol contents. Whereas the treatment of Greencal, Maxifos Ca, and *A. platensis* extract, respectively, was more effective in increasing free proline as well as phenol contents.

3.5 H_2O_2 and MDA

Results in Fig. 5 obviously showed that *Fusarium* wilt disease resulted in a rise in H_2O_2 and MDA. On the other hand, it was observed that Maxifos Ca, Greencal and *A. platensis* significantly reduced the generation of H_2O_2 and MDA. Accumulation of H_2O_2 and MDA increased in *Fusarium* wilt-infected plants. Treatment of *Fusarium* wilt-infected plants with Maxifos Ca, Greencal, and *A. platensis* reduced the generation of H_2O_2 and led to a declined MDA. The results shown that the greatest effective treatments for reducing H_2O_2 and MDA were foliar spraying with Greencal.

3.6 Antioxidant enzymes activity

As shown in Fig. 6, significant rises in the activity of POD and PPO in infested pepper seedlings. Furthermore, all treatments promoted POD, PPO activities, and the greatest rates for PPO were noticed due to the application of Greencal, Maxifos Ca, and followed by *A. platensis* respectively. Application Greencal on health as well as infected plants was the best stimulator for POD and PPO antioxidant enzymes activity.

4 Discussion

Plants are exposed to many stress factors that become more severe with the increase in climate changes [47, 48]. Several scientific studies dealt with the serious destructive of *Fusarium* vascular wilt disease on many crops and vegetables [49]. Scientists focused on reducing the risk of plant diseases by using biotic and abiotic inducers to stimulate plant physiological immunity and pathogen resistance [50]. Reducing disease symptoms and severity of infection is strong and clear evidence of resistance to disease. As shown in the results of this study, the decrease in symptoms and the severity of pathological infection were a result of the use of treatments Maxifos Ca, Greencal, and *A. platensis* extract, where Maxifos Ca and Greencal recorded high protection and lowest PDI, then came next *A. platensis* extract. These results can be explained by that green Maxifos Ca containing calcium phosphate, where calcium plays a major role in the formation of strong cell walls to prevent the penetration of fungus and the failure of the disease cycle, and phosphorus participates in many enzymatic reactions [51–53].

Table 1 Protection of Maxifos Ca, Greencal, and *A. platensis* against fusarial wilt

Treatments	Disease indicators levels					DI (disease index) (%)	Protection (%)
	0	1	2	3	4		
Control infected	0	0	2	3	5	82.5	0
Maxifos Ca	4	3	2	1	0	25	69.6
Greencal	3	3	3	1	0	30	63.63
A. platensis	3	3	2	2	0	37.5	54.5

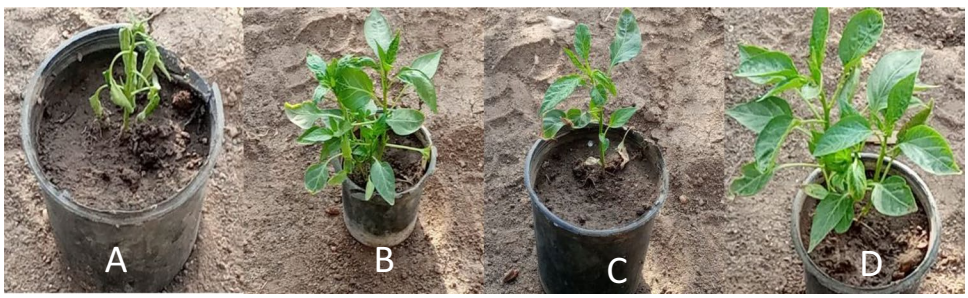

Fig. 1 Symptoms of wilt disease **A**-untreated infected, **B**-infected treated with Greencal **C**-infected treated with *A. platensis* and **D**-infected treated with Maxifos Ca

Several mechanisms have been postulated to support fungal growth inhibition by calcium phosphate [54, 55]. The toxicity of phosphite to phytofungal was due to an increased level of inorganic polyphosphate, which is known to inhibit key phosphorylation reactions in phytopathogenic fungi [56, 57]. Also, calcium phosphite is involved in activating plant defense response against many fungal pathogens [58]. On the other hand, the treatment of Greencal had a strong effect on reducing the severity of *Fusarium* wilt symptoms due to the presence of *A. nodosum* extract. These results are supported by many studies [59–61], where they reported that *A. nodosum* has antifungal effects. Marin algae secrete certain substances that include some sugars, amino acids, organic acids, as well as pathogen-inhibiting substances [62, 63]. Recently, scientific reports have proven that these vital compounds extracted from algae spread in the soil adjacent to plant roots or through leaves and are considered the most powerful biofertilizers [64–66]. Application of Greencal caused a significant improvement which the most effective treatment for recovering plant (shoot and root lengths) followed by Maxifos Ca and came next algal extract *A. platensis*, which indicates a strengthening of the plant's structural immunity and a decrease in the destructive impacts of phytopathogens. Our results are similar to the heavily studies [4, 55]. It was detected that *Fusarium* wilt-infected plants pretreated with Greencal and *A. platensis* showed encouraging disease recovery. These previous results are similar to the study of [26]; they described that the application of marine algae enhanced plant vegetative growth by polysaccharides creation. The use of *A. nodosum* to improve pepper growth has been suggested as a prospective management performance in plant yield enrichment [56]. These findings are in line with those reported by [34], who establish that treated plants with algal extract significantly enhanced their all morphological criteria. The rise in plant growth and crop with algae might chiefly be due to the release of plant nutrients and the plant growth regulators [57]. The Ca and boron deficiency in plants caused alterations in growth, physiological, biochemical, and yield attributes due to which fruit productivity gets reduced [67]. *Fusarium* wilt disease leads to a failure to capture light and carry out the photosynthesis process and imbalance in the formation of carbohydrates and proteins [68–70]. The results of this study showed an imbalance and a severe deficiency in the content of chlorophyll

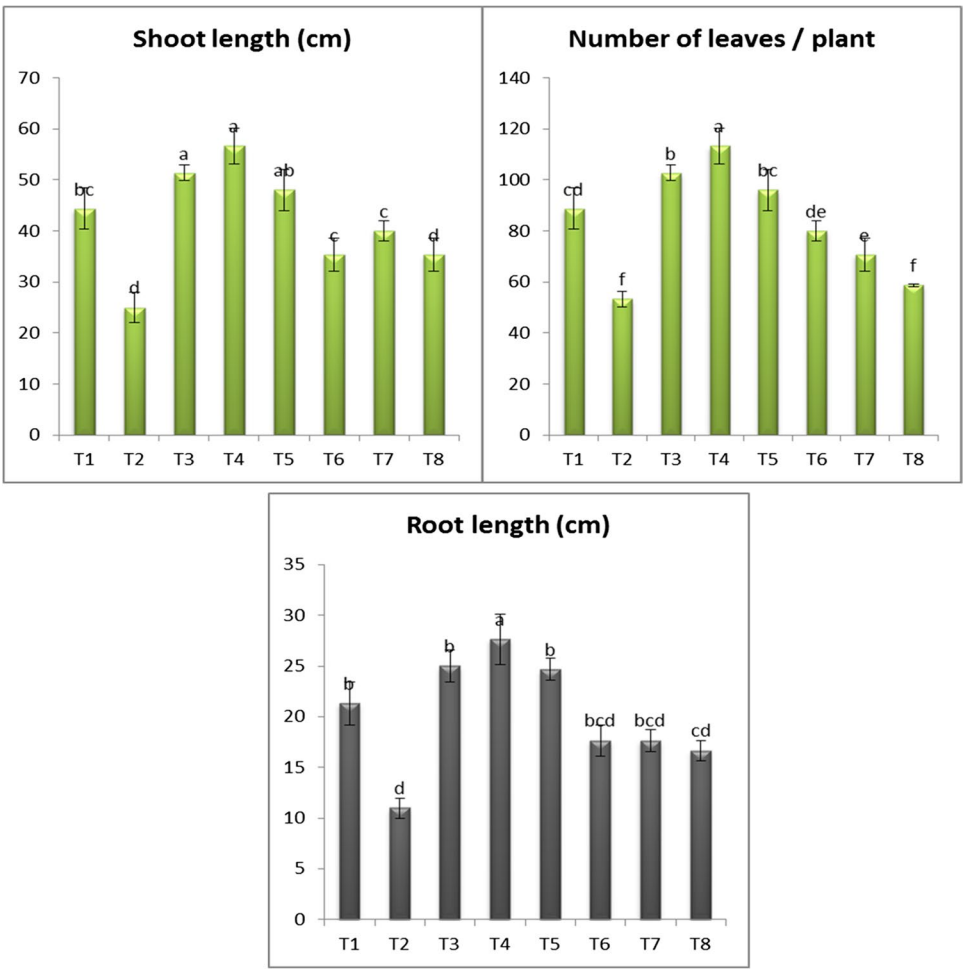

Fig. 2 Effect of Maxifos Ca, Greencal, and *A. platensis* on growth markers. (Data represent mean ± SD, $n = 3$). T1-control healthy, T2-control infected, T3-healthy and Maxifos Ca, T4-health and Greencal, T5-healthy and *A. platensis*, T6-infected pepper and Maxifos Ca, T7-infected pepper and Greencal, and T8-infected pepper and *A. platensis*)

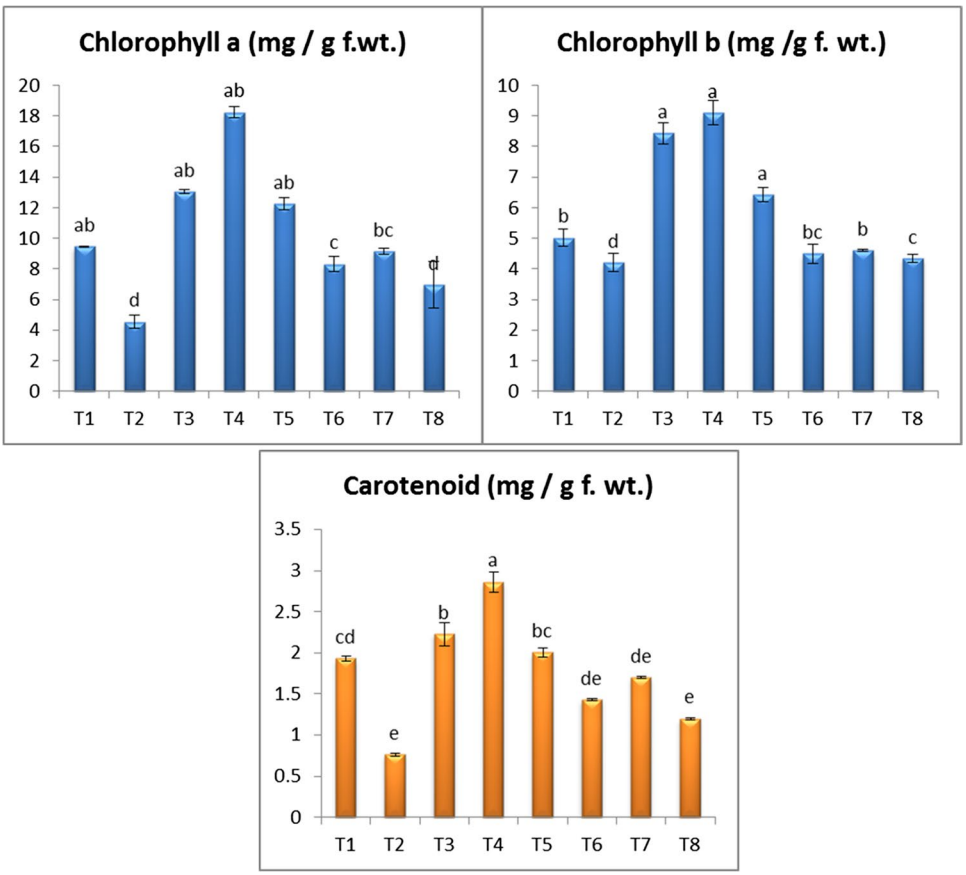

Fig. 3 Effect of Maxifos Ca, Greencal, and *A. platensis* on photosynthetic pigments. (Data represent mean ± SD, $n = 3$). T1-control healthy, T2-control infected, T3-healthy and Maxifos Ca, T4-health and Greencal, T5-healthy and *A. platensis*, T6-infected pepper and Maxifos Ca, T7-infected pepper and Greencal and T8-infected pepper and *A. platensis*)

and carotene pigments, and these results are consistent with many previous studies. It is worth mentioning in this study that the application of Maxifos Ca, Greencal, and *A. platensis* extract led to a significant improvement in the content of chlorophyll and carotene pigments. The present data reported that Greencal was the greatest effective treatment to enhance plants' levels of Chl a, b, and carotenoids of both healthy and *F. oxysporum*–infected plants These results can be explained by the biological role of Greencal, which contains *A. nodosum* extract in addition to calcium and boron elements that work to raise stress and stimulate plant immunity [64]. The increase of proline avoids damage to the photosynthesis pigments by catching the free radicals that trigger the destruction and failure of the photosynthesis process [71, 72]. Plants are forced to increase the level of proline and phenol after the occurrence of fungal infection to defend

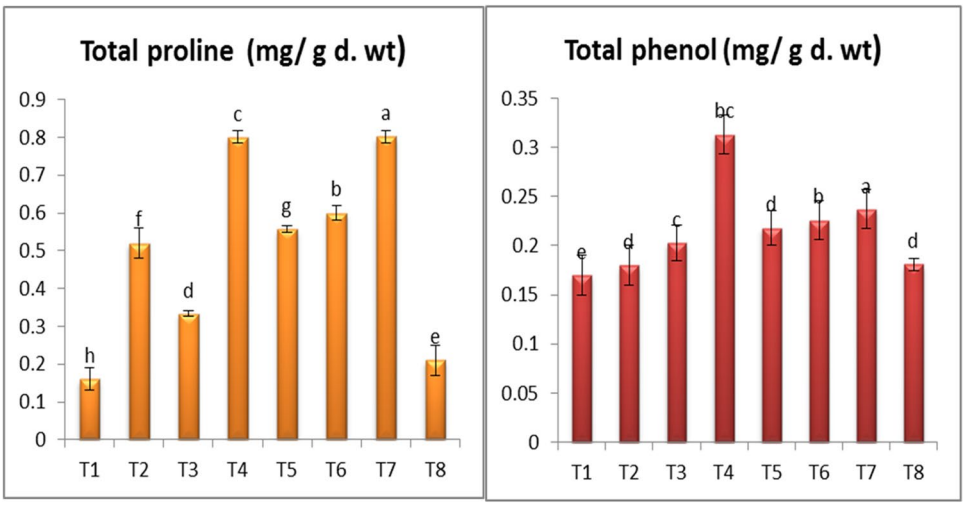

Fig. 4 Effect of Maxifos Ca, Greencal, and *A. platensis* on free proline and total phenol. (Data represent mean ± SD, $n = 3$). T1-control healthy, T2-control infected, T3-healthy and Maxifos Ca, T4-health and Greencal, T5-healthy and *A. platensis*, T6-infected pepper and Maxifos Ca, T7-infected pepper and Greencal and T8-infected pepper and *A. platensis*)

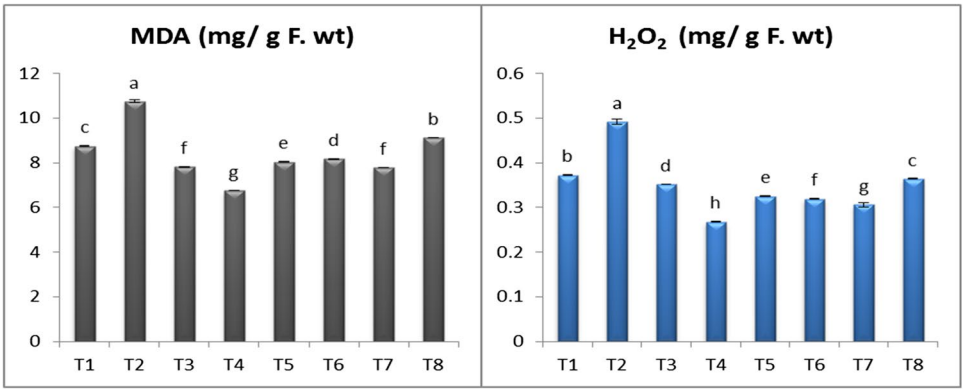

Fig. 5 Effect of Maxifos Ca, Greencal, and *A. platensis* on H_2O_2 and MDA. (Data represent mean ± SD, $n = 3$). T1-control healthy, T2-control infected, T3-healthy and Maxifos Ca, T4-health and Greencal, T5-healthy and *A. platensis*, T6-infected pepper and Maxifos Ca, T7-infected pepper and Greencal and T8-infected pepper and *A. platensis*)

against the risk of oxidative explosion and capture free radicals [73, 74]. The results of this study showed that Greencal, Maxifos Ca, and *A. platensis* extract triggers an increase of free proline and phenol content that confirms the occurrence of high resistance against the *Fusarium* wilt disease in agreement with other heavily studies [72, 75]. Phenols demonstration an vital role in scrubbing and capturing free radicals, that resulted to minimize oxidative stress in pepper plants [76]. The accumulation of phenolics in pepper plants acts as an adaptive strategy against *Fusarium* vascular wilt disease [77, 78]. Oxidative stress caused by *F. oxysporum* led to severe interruption to plant cells and the proliferation of the contents of MDA and H_2O_2 in the leaves of pepper plants. These results are in agreement with [79, 80]. Supplementation of diseased plants with Maxifos Ca, Greencal, and *A. platensis* respectively reduced the generation of H_2O_2 thus resulting in a MDA declined. The results exposed that the most effective treatment in reducing H_2O_2 and MDA was foliar spraying with Greencal all the way through accumulative antioxidants that hunt reactive oxygen species and avoid plant membranes against oxidative stress [49].

5 Conclusion

In conclusion, Maxifos Ca, Greencal, and *A. platensis* caused a significant increase in all aspects of the pepper plant. Maxifos Ca and Greencal developed in recovering growing, chlorophyll contents, proline, phenolic compounds, and antioxidant activity of pepper plant. A clear promotion in the resistance of *F. oxysporum* and promotion cell metabolism, suggesting the growth suppression and regulation by Maxifos Ca®, Greencal®, and *A. platensis*. Accordingly, Maxifos Ca® and Greencal® are promising agents for potential in the agricultural application and as a smart biological control against pepper *Fusarium* wilt**.** The current study recommends the use of Greencal® (a unique formulation of seaweed Ascophyllum nodosum with calcium), *Arthrospira platensis*, and Maxifos Ca® (contains calcium in the form of phosphite, which increases plant resistance to biotic and abiotic stresses as well, increases vegetative growth and supports immune responses). Therefore, Greencal® and Maxifos Ca® consider therapeutic nutrients to improve immune

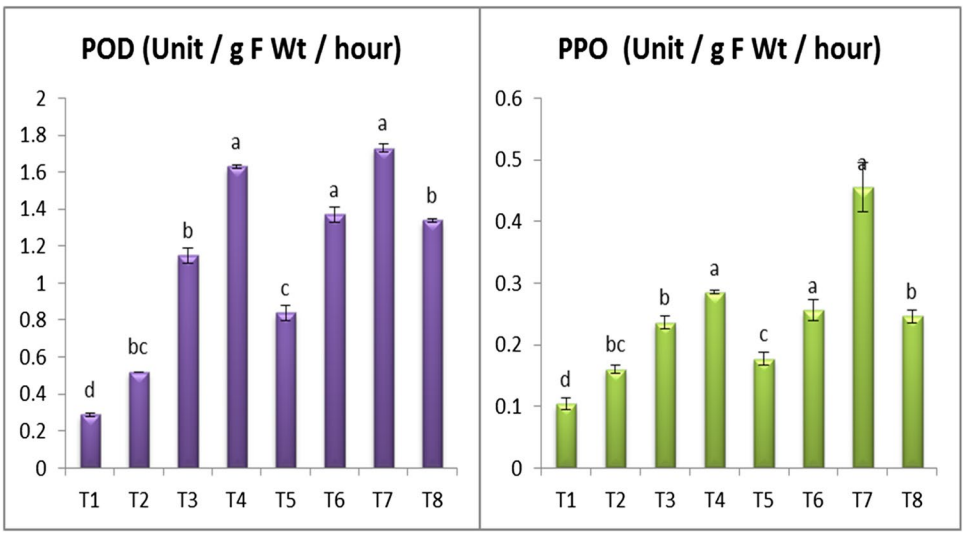

Fig. 6 Effect of Maxifos Ca, Greencal, and *A. platensis* on POD and PPO. (Data represent mean ± SD, $n = 3$). T1-control healthy, T2-control infected, T3-healthy and Maxifos Ca, T4-health and Greencal, T5-healthy and *A. platensis*, T6-infected pepper and Maxifos Ca, T7-infected pepper and Greencal and T8-infected pepper and *A. platensis*)

responses and enhance plant health against fungal wilt disease to reduce the use of chemical pesticides.

Acknowledgements The authors are grateful to Eng. Mahmud M. Elsayed, for his help during the study. The authors are also grateful to Al-SALAM International for Development & Agriculture Investment, Egypt for the financial and technical support offered during this work. The authors are also grateful to MAFA-VEGETAL ECOBIOLOGY-Spain. The authors would like to thank the Botany and Microbiology Department, Faculty of Science, Al-Azhar University for promoting this research.

Author contribution A. M. A., S.M.E, M. M. A., and M. S. A.—methodology; A. M. A., S.M.E, M. M. A., and M. S. A.—software; A. M. A and M. S. A—formal analysis. A. M. A. and M. S. A.—investigation. A. M. A. and M. S. A—resources. A.M.A. and M.S.A.—data curation. A.M.A., M.S.A., and S.M.A.—writing original draft preparation. A. M. A., M.M.N., and M. S. A.—writing review and editing. A. M. A., M.M.N, and M. S. A—supervision. A. M. A., M. M. A, and all authors have read and agreed to the published version of the manuscript.

Funding Open access funding provided by The Science, Technology & Innovation Funding Authority (STDF) in cooperation with The Egyptian Knowledge Bank (EKB).

Data availability All data and materials are viable**.**

Declarations

Ethics approval and consent to participate All authors approved.

Consent for publication All authors agree to the publication.

Competing interests The authors declare no competing interests.

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