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PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

OPEN ACCESS Pakistan Journal of Biological Sciences

ISSN 1028-8880
DOI: 10.3923/pjbs.2021.618.628

Research Article
Bio-Efficacy of Two Algae against *Bruchidius incarnatus*, Physiological and Cytogenetic Effects on *Vicia faba*

[1]Rania S. Rashwan and [2]Maissa M. Morsi

[1]Department of Plant Protection, Faculty of Agriculture, Ain Shams University, Cairo, Egypt
[2]Department of Botany, Faculty of Women for Art, Science and Education, Ain Shams University, Egypt

Abstract
Background and Objective: The bean seed beetle *Bruchidius incarnatus* is a major insect pest for stored grains that causes great economic damage. The investigated research aimed to evaluate the efficacy of two species of algae: *Fucus vesiculosus* and *Spirulina platensis* as natural alternative pesticides against *Bruchidius incarnatus* stages. **Materials and Methods:** The efficacy of two tested algae with amounts of 0.25, 0.35 and 0.50 g were evaluated on *B. incarnatus* stages. The activities of some biochemical components were assayed to determine the algae effect. Seed germination and growth parameters were studied. **Results:** *F. vesiculosus* caused higher potent on larval and adult stages than *S. platensis*. Antioxidant enzymes Glutathione Peroxidase (GPX) and Superoxide Dismutases (SOD) in treated adults have reached the highest level when compared with control. Some biochemical components in adults were affected also by algae treatment. Treatment with two algae caused stimulation of seedling and germination development. On the other hand, both types of algae occurred an expansion in the mitotic index and low levels of abnormalities. **Conclusion:** Both types of algae are considered a promising Bio-insecticide in controlling stored grain pests and it may be considered Bio-eco-friendly in pest management.

Key words: *B. incarnatus*, *F. vesiculosus*, *S. platensis*, seed germination, growth, infested seeds, algae treatment

Citation: Rashwan, R.S. and M.M. Morsi, 2021. Bio-efficacy of two algae against *Bruchidius incarnatus*, physiological and cytogenetic effects on *Vicia faba*. Pak. J. Biol. Sci., 24: 618-628.

Corresponding Author: Rania Rashwan, Department of Plant Protection, Faculty of Agriculture, Ain Shams University, Cairo, Egypt

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Grains are the most common food for a human that consist of numerous daily needed contents. However, various insect pests, known as storage grain pests, can attack them. These insects cause a reduction in the quality and quantity of these important crops. Chemical pesticides widely used against plant pests may have several shortcomings. Indeed, excessive use of chemical pesticides gives rise to insect resistance. For instance, pyrethroid and organophosphorus considered the most applicable classes of pesticides in agriculture, cause difficulties in controlling pests[1]. Consequently, looking for a new safer alternative that is less harmful and biodegradable to nontoxic substances in the environment, is attracting the attention of researchers everywhere throughout the world.

Broad Bean (*Vicia faba* L.) is planting on a large scale in the Arab Republic of Egypt and considered the main food for human. This attacked a major crop by various insect pests leads to reduce the economic values. *Bruchidius incarnatus* is recorded as the most destructive insect pest. Feeding on the contents and the embryos of seeds can reduce the percentage of germination leading thus to low quality and quantity of crops[2].

Spirulina platensis belongs to photosynthetic organisms, which has radical scavenging properties and are antioxidant models[3]. The microalgae *Spirulina platensis* and *Fucus vesiculosus* are widely used in controlling many pests[4]. Algae improve growth, physiology and act as an antioxidant system of *Vicia faba*[5]. Moreover, they help in increasing soil fertility and used as bioremediation methods for the de-pollution of heavy metals because of their low cost and high efficiency[6].

Mitotic index is a marker of cytotoxicity and is considered as a power parameter of cell division. Marcano *et al.*[7] stated that cytotoxicity could be described as a depression in mitosis and as an extension in the fraction of cells with c-mitosis, sticky chromosomes, multi-polar anaphase and laggards. Extracts of several plants showed chromosomal damage and antimitotic properties[8]. A dusting treatment with Black cumin and *Lupinus termis* powder on *Vicia faba* seeds caused a significant decline in the mitotic scale and a significant value of abnormal mitosis[9]. Genotoxicity is explained as different kind of DNA lesions and mutations, expanding from gene to number and morphological chromosome variations. Moreover, both the creation of micronuclei in interphase cells and the presence of various mitotic aberrations establish the genotoxic influence.

In this study, our objective was to assess the insecticidal activity of two types of algae known as *Spirulina platensis* (cyanobacterium) and *Fucus vesiculosus* (brown alga) against bean seed beetle *Bruchidius incarnatus* as a Bio-insecticide and to follow the treatment effects on seedling and cytogenetic parameters of a seed plant.

MATERIALS AND METHODS

Study area: The study was carried out during 2019 at the Department of Biology, Faculty of Science, Taif University, KSA.

Culture of bean seed beetle (*B. incarnatus*): The variety of bean seeds used in the study was Var.Giza-716, it obtained from Field Crops Institute Research, Egypt. The insect was obtained from infested seeds, which collected from the local seed market. Plastic jars (25 cm height *10 cm width) were prepared for insect rearing. Jars included infested grains by a mentioned insect. To prevent insect escape while still maintaining ventilation, muslin cloths were used to cover jars. The optimum conditions for rearing in the laboratory were constant during all the experiments (27$\pm$1°C and 75% RH)[10]. Collected adults used in experiments were ranged from 1-2 days old.

Preparing of algae extracts and their sources: Brown alga (*F. vesiculosus*) was obtained from a local market in Egypt, while cyanobacterium (*S. platensis*) tablets were purchased from (MLM) in Malaysia. The electrical grinder was used to get powder for two algae *S. platensis* and *F. vesiculosus*. Treatment amounts of 0.25, 0.35 and 0.50 g were utilized to examine the effect of both algae.

Bioassay of two tested algae on adult stage: Three hundred grams of un-infested grains were harmony blended with tested algae powder in a shaker for 2 min to a congruity covering on the seed surface[11]. Treated grains were introduced to adults to allow feeding. Three replicates were treated for each amount (20 adults/replicate). Untreated grains were used as control. Percentages of corrected mortalities were calculated and recorded after 3 days according to Abbott[12].

Bioassay of two tested algae on egg and larval stage: Three hundred grams of infested grains with egg stage only were blended slightly with algae powder for 2 min. Egg hatchability was examined under a microscope to calculate egg hatchability. Three replicates were treated (30 egg/replicate). Larval mortalities were also recorded (20 larvae/replicate). Untreated grains were considered as control.

Biochemical assessment

Preparation of samples: For measuring the biochemical components in insect tissue after treatment with LD_{50} of two types of algae, dead adults were put in Eppendorf tubes. Teflon homogenizer (Cairo, Egypt) used with distilled water in the homogenization process. Tubes were centrifuged at 450 rpm for 10 min at 5°C. The produce from this process which is known as supernatant of treated and untreated (control) was immediately assayed to determine the activities of total soluble protein, Acetylcholinesterase, phosphatases (acid and alkaline) and two kinds of the antioxidant enzyme.

Determination of Total Soluble Protein (TSP): The total soluble protein of insect was conducted as the method followed by Gornall *et al.*[13]. A volume of 0.2 mL of adult homogenate was added to 5 mL of Biuret reagent (USA) and incubated for 30 min at 20-25°C.

Determination of Acetylcholinesterase (AChE): Measuring for Acetylcholinesterase activity was estimated following the reported method of Simpson *et al.*[14].

Measurements of alkaline phosphatases and acid phosphatase enzymes (AlkP and AcP): Determination of Acid Phosphatase (AcP) and Alkaline Phosphatase (AlkP) activities were carried out according to the method described by Powell and Smith[15].

Measurements of Glutathione Peroxidase (GPX): The activity of the Glutathione Peroxidase (GPX) was estimated with H_2O_2 as substrate as reported by Paglia and Valentine[16].

Measurements of the activity of Superoxide Dismutase (SOD): Total SOD activity was measured according to the method reported by Marklund and Marklund[17] where the total SOD activity was measured.

Physiological and cytogenetically studies

Seed germination and growth characters: Un-infested grains of bean (*Vicia faba*) were chosen with the same size and colour, the surface of the seed was sterilized in 5% ethanol for 15 min and washed three times with autoclaved distilled water.

Three seeds lots in three replicas for each (ten Un-infested seeds, ten infested seeds and ten treated Infested-seeds with two types of algae) were put on filter papers in a diameter of 9 cm of Petri dishes and kept in laboratory conditions for 10 days.

Five parameters (Percentage of seed Germination, Germination Rate (GR), Vigour Index of seeds (VI), Relative Germination Ratio (RGR) and percentage of germination Inhibition (I)) were measured after 8 days of incubation[18]. Root and stem length of bean seedlings were measured after 10 days of sowing.

Cytogenetic study: Filter papers were wet with tap water to germinate bean seeds (control, infested and treated seeds) at laboratory temperature 25°C for 48 hrs. After reaching 2 cm of length, roots were cut off, fixed in Carnoy's fixative (1:3 acetic alcohol), hydrolyzed in 0.1 N HCl. squashed and stained with Feulgen squash technique. Five slides were prepared temporary for control, treatments and infested seeds. One thousand cells were tested per slide. Phase index and Mitotic index, Chromosome abnormalities were examined at magnification 100x objective and 15x eyepiece Olympus microscope camera (Leica, Germ.). Slides were examined by using oil immersion.

Assay of micronucleus: The amount of one thousand interphase cells from five slides were estimated for micronuclei assay and expressed as MN 1000^{-1} cell. MN efficiency was recorded from the Meristematic (M) and First generation (F1) cells of the tips of bean root where it compared by a treated/control (T/C) ratio[19].

Data analysis: Statistical analysis was performed using the SAS program[20]. The Least Significant Differences (LSD) were calculated using the Duncan option in the program. Profit analysis was determined to calculate LD_{50} through a software computer program.

RESULTS

Susceptibility of *B. incarnatus* stages to treatment with two algae *Spirulina platensis* and *Fucus vesiculosus*: Obtained results show the effect of two tested algae (*S. platensis* and *F. vesiculosus*) on insect mortality. High significant differences have been reported in mortality percentages between two algae compared with control. Given that no egg mortalities were observed, the treatment with the two algae did not have a notable effect on the hatching of eggs. Different mortality percentages of larvae have been shown when are treated with two algae with three amounts (Fig. 1a). The highest mortalities were recorded at 0.5 g. Mortality percentages were 43 and 89% for two tested algae *S. platensis* and *F. vesiculosus*, respectively.

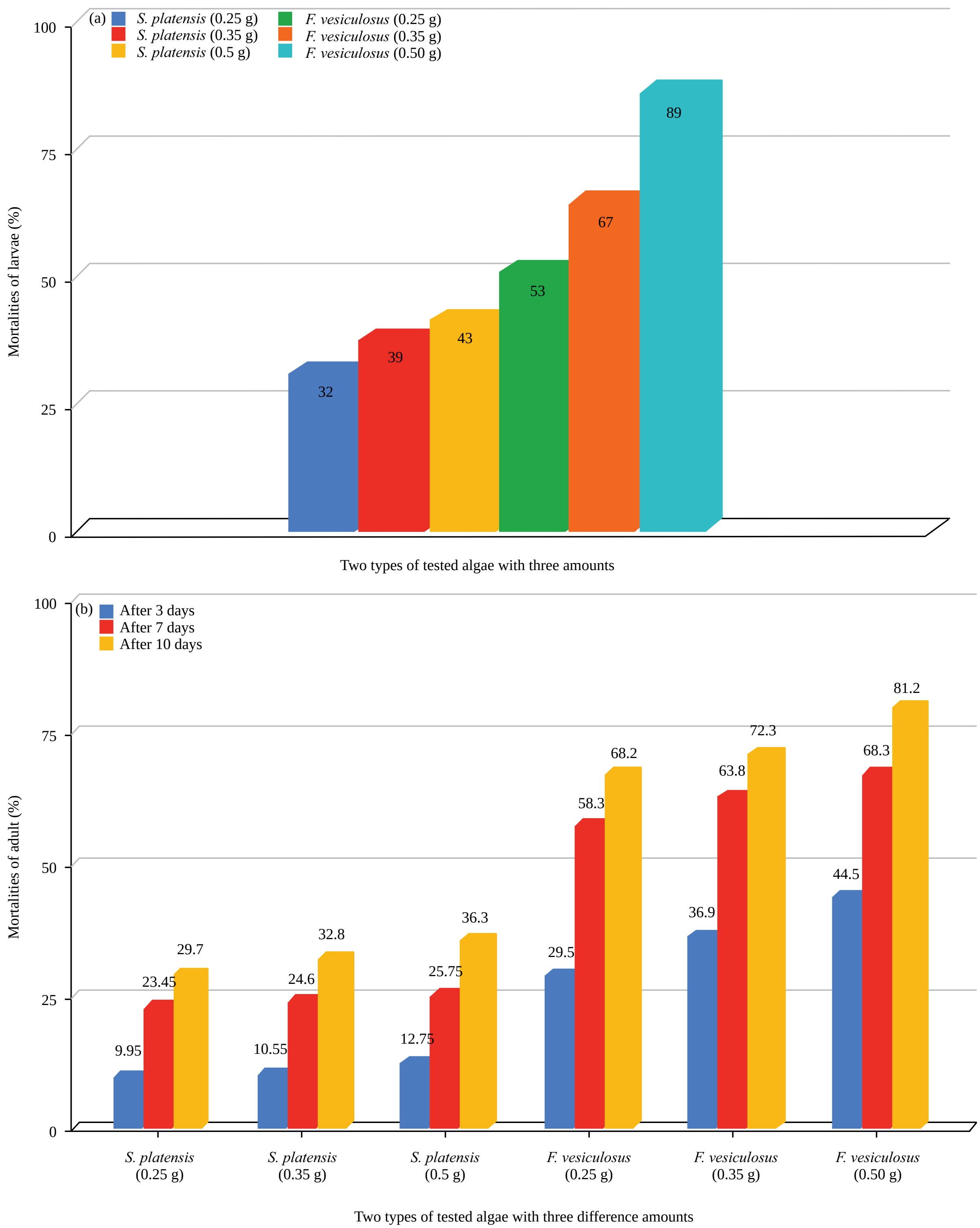


Fig. 1(a-b): Percent Mortalities of treated *B. incarnatus* (a) Larvae and (b) Adults with *S. platensis* and *F. vesiculosus*

Adult mortalities were lower when seeds treated with *S. platensis* than *F. vesiculosus*. Treatment with *S. platensis*, recorded 29.7, 32.8 and 36.3% of mortalities after 10 days when treated respectively with amounts of 0.25, 0.35 and 0.5/300 g. We obtained 68.2, 72.3 and 81.2% with amounts of 0.25, 0.35 and 0.5/300 g, respectively when treated with *F. vesiculosus* (Fig. 1b).

Table 1: Measurements of some biochemical components (mean±SE) in the treated adults *Bruchidius incarnatus* by two types of algae

Treatments	Total soluble protein (mg g^{-1} b.wt.)	Acetylcholinesterase (mmol min^{-1} mL^{-1})	Acid-phosphates measurement (µg phenol g^{-1} b.wt. min^{-1})	Alkaline phosphates measurement (µg phenol g^{-1} b.wt. min^{-1})	Antioxidant enzymes (U mg^{-1} protein) Glutathione peroxidase (GPX)	Antioxidant enzymes (U mg^{-1} protein) Superoxide dismutase (SOD)
F. vesiculosus	0.772 ± 0.23^{b}	256.28 ± 0.32^{c}	0.011 ± 0.21^{c}	0.043 ± 0.21^{b}	8.36 ± 0.25^{a}	6.35 ± 0.12^{a}
S. platensis	1.265 ± 0.12^{b}	386.24 ± 0.24^{b}	0.027 ± 0.12^{b}	0.053 ± 0.16^{b}	6.27 ± 0.13^{b}	4.28 ± 0.36^{b}
Control	2.913 ± 0.02^{a}	426.35 ± 0.12^{a}	0.046 ± 0.02^{a}	0.076 ± 0.04^{a}	2.63 ± 0.12^{c}	0.67 ± 0.23^{c}
LSD	1.32	38	0.012	0.02	2.03	1.53

*Different superscripted letters ^{a,b,c} between columns demonstrated significant differences when using the LSD test

Effect of the tested two algae on some biochemical components of *Bruchidius incarnatus* adult

Total Soluble Protein (TSP): The efficiency of the tested algae was expressed as LD_{50} values. These values were estimated from the plotted toxicity regression lines of mortality percentages. LD_{50} Values for *S. platensis* and *F. vesiculosus* were recorded after 3, 7 and 10 days. The estimated LD_{50} values for S. *platensis* were 3.62, 3.1 and 1.016 g, while for *F. vesiculosus* 0.69, 0.086 and 0.004 gm respectively for 3, 7 and 10 days.

Results in a Table 1 investigated significant differences between the two algae. Algae have significantly reduced the total soluble protein in treated insects.

The value of total protein in the supernatant of the homogenate's adults recorded at 0.772 and 1.265 mg g^{-1} b.wt. respectively when treated with *F. vesiculosus* and *S. platensis*. For control, it was 2.913 mg g^{-1} b.wt.

Acetylcholinesterase: As shown in Table 1 adults that exposure to treated grains with *F. vesiculosus* and *S. platensis*, a decreased acetylcholinesterase activity levels to 256.28 and 386.24 as compared to 426.35 in the control. These results are supported with statistical analysis which demonstrated significant differences between two types of algae and control.

Acid phosphatase and alkaline phosphatase: The data of Table 1 indicated that the two tested algae reduced the activity of acid phosphatases compared to untreated adults. The mean values of acid phosphatase activities in the supernatant of the homogenate adults reached 0.011 and 0.027 mg g^{-1} b.wt. when treated with *F. vesiculosus* and *S. platensis*, respectively. In control, it was 0.46 mg g^{-1} b.wt.

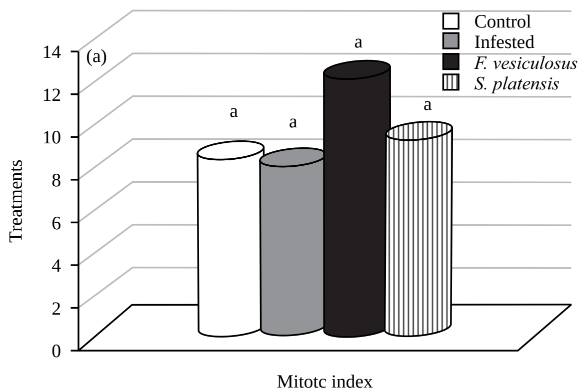
Results also revealed that treatment with two algae decreases alkaline phosphatase activity. Indeed, the mean values of alkaline phosphatase enzymes activities reached 0.043 and 0.053 mg g^{-1} b.wt., respectively for *F. vesiculosus* and *S. platensis* while for control it was 0.076 mg g^{-1}. Statistical analysis proved a highly significant difference between two algae and control.

Activities of antioxidant enzymes: Glutathione Peroxidase (GPX) and Superoxide Dismutase (SOD): As shown in Table 1, GPX and SOD activities were increased in *B. incarnatus* after treatment with the two algae. High values of GX activity were obtained in adults treated with the two algae compared to that obtained in control. It was 8.36 and 6.27 after treatment with *F. vesiculosus* and *S. platensis,* respectively. While in control, it was 2.63. Likewise, an increase in SOD activity was recorded in adults treated with the two algae. Indeed, the SOD activity value increased from 0.67 in control to 6.35 and 4.28 after treatment with *F. vesiculosus* and *S. platensis,* respectively.

Effect of the two tested algae on physiological and cytogenetically parameters of *Vicia faba* plants

Seed germination and seedling growth: The infested seeds scored a decline in the percentage of germination compared to the un-infested (control). Normal germination was recorded after treatment of seeds with *S. platensis*, *F. vesiculosus* previously infested (Table 2). Seed germination and seedling growth parameters were highly estimated in both *S. platensis* and *F. vesiculosus* compared with infested beans (Table 2). Otherwise, like the control, no inhibition of germination (I) in the treated seeds compared with the infested beans that showed high inhibition.

A significant depression in roots, shoots and plant lengths were found in the infested seeds compared to control. The root length of the untreated infested-seeds is 1.25 cm in comparison with 2.4 cm for control (un-infested). Furthermore, the root lengths were 2.59 and 2.55 cm after handling with *F. vesiculosus* and *S. platensis* respectively. While the highest shoot length reached 5.63 and 4.04 cm with *F. vesiculosus* and *S. platensis* treatments as compared to control. These results of root and shoot lengths gave rise to plant length increment (Table 2).



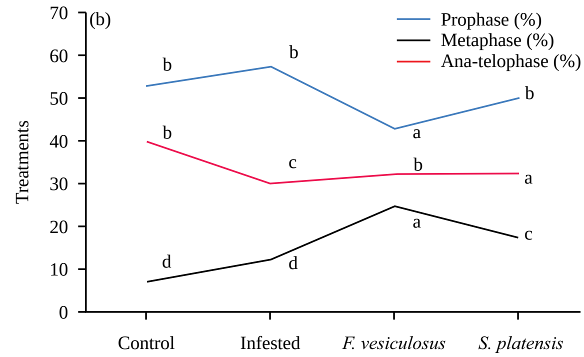


Fig. 2(a-b): Cytotoxic effect of two tested algae on *Vicia faba* compared with infested seeds
(a) Mitotic index and (b) Phase index in mitotic cells

Table 2: Seed germination and seedling growth of *V. faba* treated with *S. platensis* and *F. vesiculosus* in comparison with un-infested and infested grains

	Treatments (means±SD)					
Parameters	Control (un-infested seeds)	Infested grains	*F. vesiculosus*	*S. platensis*	F-value	LSD
Seed germination						
GP	93±1^{a}	40±2^{b}	93±2^{a}	92±1^{a}	298.8	52
GR	23.25±0. 25^{a}	10.00±0. 5^{b}	23.25±0. 5^{a}	23.00±0. 25^{a}	298.7	1.3
RGR	1±0. 1^{a}	0.43±0. 025^{b}	1.00±0. 025^{a}	0.98±0. 015^{a}	264	0.06
Vigour index	86.65±1^{a}	35.25±2^{c}	84.78±2^{ab}	85.56±1^{a}	274	44
I	0^{c}	56.98±2.15^{a}	0^{c}	1.07±0. 015^{b}	340	5.4
Seedling growth						
Root length	2.40±0. 4^{a}	1.25±0. 25^{b}	2.59±0. 09^{a}	2.55±0. 55^{a}	5.52	1.03
Shoot length	3.80±0. 4^{b}	3.50±0. 5^{b}	5.63±0. 37^{a}	4.04±0. 04^{ab}	13	1.6
Plant length	6.20±0. 8^{b}	4.75±0. 75^{c}	8.22±0. 28^{a}	6.59±0. 59^{b}	14.16	1.39

*Superscripted letters ^{a,b,c}within row appears different significant differences where p<0.05. GP: Germination percentage, GR: Germination rate, RGR: Relative germination ratio, I: Percentage of germination inhibition

Table 3: Genotoxic effect and percentage of recorded abnormalities in *V. faba* roots treated with *S. platensis* and *F. vesiculosus*

		Types of abnormalities					
	Total (%) of abnormalities	Disturbance	Irregular prophase	Stickiness	Bridge	Laggards	Fragments
Control	13.25±0.59^{d}	63.63±0.51^{b}	18.18±0.03^{b}	9.09±0.23^{c}	9.09±0.23^{c}	0^{b}	0
Infested grains	32.50±0.56^{a}	42.30±0.34^{d}	23.07±0.44^{a}	15.38±0.12^{a}	15.38±0.24^{b}	3.84±0.16^{a}	0
F. vesiculosus	27.27±0.40^{c}	66.66±1.23^{a}	12.12±0.12^{c}	9.09±0.23^{c}	9.09±0.22^{c}	3.03±0.07^{a}	0
S. platensis	30.43±0.51^{b}	60.71±0.13^{c}	3.57±0.0.15^{d}	17.85±0.12^{a}	17.85±0.24^{a}	0^{b}	0
f-value	83.90	44.6	19.5	62.5	52.6	78.9	0
LSD	1.40	2.68	3.2	4.46	4.46	126	0

*Superscripted letters ^{a,b,c}within a column recorded different significant differences where p<0.05

Cytogenetic studies

Mitotic Index (MI) and transition of mitotic phases: The percentage of mitosis (MI) in seeds treated with *F. vesiculosus* and *S. platensis* was surpassed by the control and the infested bean seeds. It reached 12.1 and 9.2%, respectively (Fig. 2a-b).

Genotoxicity study

Abnormalities: The data of Table 3 showed that the mitotic abnormalities percentage in *Vicia faba* meristematic cells was significantly higher in untreated infested-seeds (32.5%) than that in control (13.25%). However, infested seeds treated by *F. vesiculosus* and *S. platensis* exhibited lower abnormalities percentage than infested pest seeds. Different types of chromosomal abnormalities were observed as shown in Table 3. No fragments were observed in algae treatments.

Disturbance and irregular prophase were the most common types of abnormality (Fig. 3a-o). where Fig. 3a-b showed irregular prophase, (Fig. 3c): disturbed in metaphase, (Fig. 3d): disturbed in metaphase with a fragment and (Fig. 3e): disturbed metaphase with laggard chromosomes.

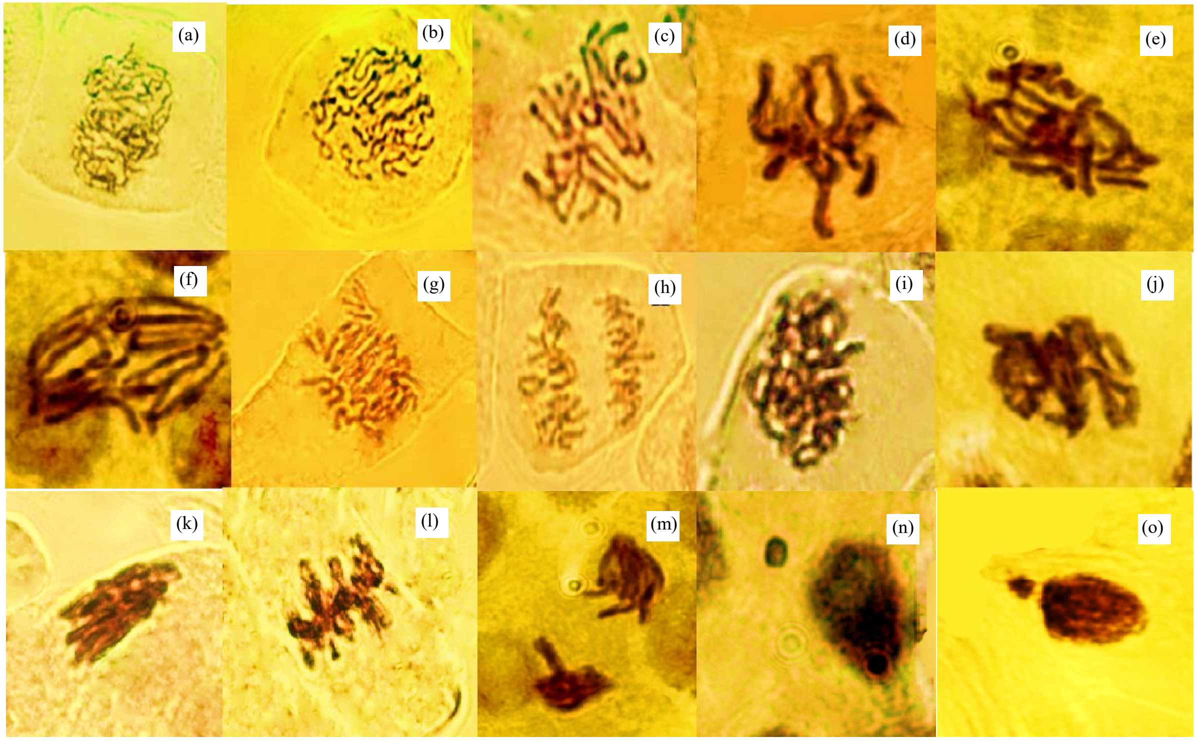


Fig. 3(a-o): Various types of chromosome abnormalities of *Vicia faba* formed by two algae and infested seeds
(a-b) Irregular prophase, (c) Disturbed metaphase, (d) Disturbed metaphase with a fragment, (e) Disturbed metaphase with laggards, (f-g) Disturbed anaphase with multi bridges, (h) Disturbed anaphase with a laggard, (i) Stickiness in prophase, (j) Sticky and disturbed metaphase, (k) Severe sickness in metaphase, (l) Sticky metaphase with fragments, (m) Severe stickiness in anaphase and (n-o) Macronucleus in interphase. Magnification: 1000X

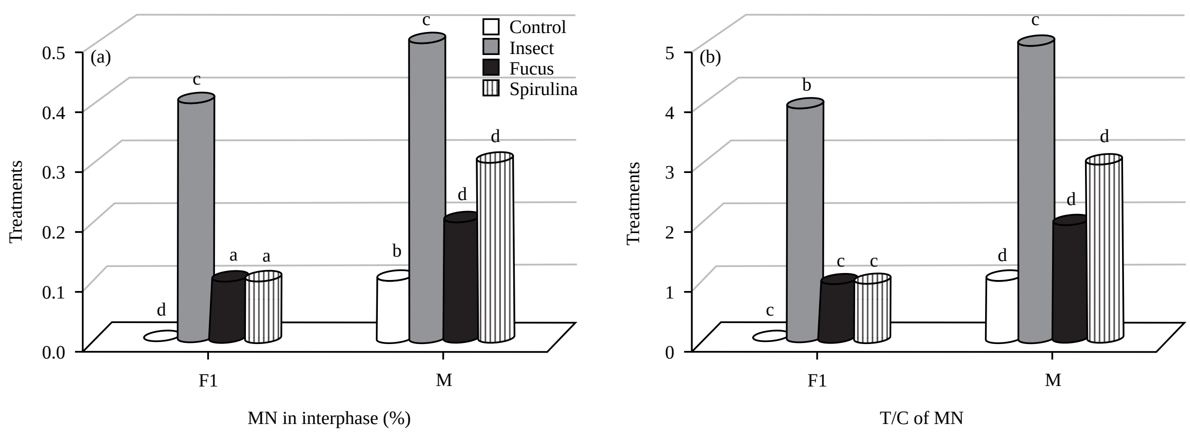


Fig. 4(a-b): Micronucleus in F1 and M cells of *Vicia faba* treated with two types of algae compared with infested bean seeds
(a) Micronucleus (%) and (b) T/C of Micronucleus

Another marked abnormality is a chromosomal bridge (Fig. 3f-g). Figure 3h showed the existence of Laggards with low percentages. Stickiness was the second popular aberration (Fig. 3i-k). where (i) exhibited stickiness in prophase, (j): sticky and disturbing metaphase, (k): severe sickness in metaphase. A rare type of abnormality observed in this study is chromosome fragmentation (Fig. 3l). Figure 3m showed also severe stickiness in anaphase. Figure 3n-o showed interphase with macronucleus.

Micronucleus assay: Micronucleus test showed low percentages after treatment with two algae compared to the infested seeds and all values were recorded low in First Generation (F1) than Meristematic (M) cells (Fig. 4a-b).

Significant differences were recorded between F1 and M for micronucleus (MN %) in interphase, also significant differences were recorded between (F1) and (M) for T/C of MN, F value = 27.2 and 186.2, respectively.

DISCUSSION

The Obtained results in this research demonstrated that increasing the number of algae increased the percentage of mortalities. It could be concluded that treated eggs were not affected by two tested algae. It is noticeable that *Fucus vesiculosus* caused a high effect on the larval stage than *Spirulina platensis*. These results are agreed with the findings of Saber[21] who recorded low mortality percentages of the treated egg of *Spodoptera littoralis*. More cover, Rashwan and Hammad[4] have recorded no effect of algae on egg hatchability of *Spodotera littoralis*.

In the same trend, Elbanna and Hegazi[22] have reported that when the second larval instar of *Culex pipiens* fed on dried algae *Padina pavonica*, they neither died nor developed ordinarily. Larvae that fed on dried algae stayed alive without growing when compared with control. The authors also have mentioned that brown algae *P. pavonica* significantly affected larval growth where it died after ten days from treatment. Treatment with *P. pavonica* inhibited larvae to change into the pupal stage. This development arrest may be due to the highest level of phenolic contents in the alga.

Besides, Riddick *et al.*[23] demonstrated that quercetin (a bioflavonoid) has been detected in some algae that have a repellent and insecticidal activity against many insects, such as plant lice insect (aphids). Our results agreed with the finding of Zubia *et al.*[24]. The authors stated that there is a specific type of tannin that is produced by brown algae known as phlorotannins. The presence of some biological components of alga as terpenoids, fatty acids and phenolic substance may cause insecticidal activity. Helmi and Mohamed[25] stated that phenols have an insecticidal effect where it inhibits the development of insect stages, where the defence mechanism in the insect is affected by phenolic components.

Zodape[26] reported that *F. vesiculosus* have could increase soil fertility by stimulating seed germination and decrease fungal diseases. Abbas[27] found that algae consist of many minerals like nitrogen, potassium, magnesium, sulphur, calcium and phosphorous as well as many other minerals. It also including proteins and vitamins, some growth regulators, carbohydrates, natural enzymes polyamines that could increase the soil fertility that helping less depending on chemical fertilizers.

Insecticidal stress might cause a reduction in protein contents[28]. Therefore, decreasing protein levels could be related to one or more factors. The same authors indicated that reduction of the protein contents resulted in the presence of amino acids that provide the insect with energy which in turn peak down proteins used recovering insecticidal stress. Moreover, decreasing protein-synthesizing could also be associated with a reduction in nucleic acids levels.

Two types of algae decreased and inhibited acetylcholinesterase activity, which reflects the increased level of acetylcholine in post synapses. This accumulation causes synaptic nerves still on, led to insect paralysis. It has been reported that treated cotton leafworm with some plant extracts caused a notable decrease in the acetylcholinesterase enzyme activity[29]. Al-barty *et al.*[30] found that exposure of *Oryzaephilus surinamensis* beetles to milled wheat grains treated with different concentrations of garlic oil decreases acetylcholinesterase activity.

Treatment with two algae affected the activity of acid phosphatase and Alkaline phosphatase. Abdel Aziz[31] have studied the efficacy of two insecticides on *S. littoralis*. They reported that the larvae treated with the LC_{50} of the tested compounds caused a variable reduction in alkaline phosphatase activity.

Superoxide dismutase is the most powerful antioxidant enzyme. In insects treated with algae, SOD activity reached high levels. Superoxide radical in insect tissues is induced when treated with algae. GPX activity significantly increased in response to algae treatments that induced oxidative stress in tissues of *B. incarnatus*. Under physiological conditions, intracellular antioxidant enzymes, such as GPX and SOD eliminate Reactive Oxygen Species (ROS). These enzymes play a crucial role in the oxidative stress defences of the cell by preventing and fixing damage occurred by ROS. Dubovskiy *et al.*[32] stated that antioxidant enzyme activities were affected by pesticides. Rashwan *et al.*[33] have demonstrated that treated adults of *S. oryzae* with radiant led to oxidative stress where superoxide dismutase and catalase activities were increased compared to untreated insects. Copping and-Menn[34] reported that an increase in SOD activity might be due to an increase in ROS. Das[35] showed that GPX has a protective effect when an insect was exposed to oxidative stress and increased its life cycle. In general, the two tested algae *F. vesiculosus* and *S. platensis* influence the biochemical components that were measured in *B. incarnatus* adults as responding factors.

Germination inhibition in untreated infested-seeds compared with *S. platensis* and *F. vesiculosus* treated seeds, maybe due to a direct predator action of insect on seeds. By feeding on the seed, the insect removes many endosperm contents, induces death of the embryo leading to seed germination inhibition. Fox *et al.*[36] demonstrated a depression in the germination of *Acacia greggii* and *Parkinsonia florida* after infestation with one or more larvae and exhaustion of cotyledon, which eventually caused a gradual decrease in plant growth.

In general, infested seeds showed a significant inhibitory effect on mitotic activity. The decline in MI could be due to a cell cycle arrest at the G2 phase or suppression of DNA synthesis. It has been reported that inhibition of the cell cycle progression could be explained by the damage of chromosomes due to special proteins introduced by the pesticides[37]. The present study indicates that *F. vesiculosus* and *S. platensis* are safe and effective biofertilizers, promoting *Vicia faba* growth by increasing cell division. However, another study has demonstrated that cell division increment is referred to the length of telomeres, since the end of chromosomes was protected by nucleoprotein structure and its efficacy encourages entry into cell cycle division[38].

For mitotic stages transition, the prophase stage showed a higher frequency than other phases. The prophase stage resulted from the retardation of the spindle fibre structure. Algae treatment gave a highly significant number of prophase cells accompanied by its highly significant increase of mitotic indices. However, the checkpoints ("metaphase-checkpoint "and or anaphase-promoting complex) infiltrated a large number of prophase cells, low percentage of metaphase cells and normal percentage of Telophase.

A high percentage of abnormalities was demonstrated in infested seeds compared to control and infested seeds treated by *F. vesiculosus* and *S. platensis.* Nwakanma and Okoli[39] illustrated that the spindle failure and nucleotoxic action of the treatments are the main cause of chromosomal abnormalities.

Different types of abnormalities were recorded, among them were disturbance and irregular prophase with high percentages. Pankaj and Kumari[40] stated that the disturbance of the spindle apparatus or the prohibition of the cell cycle at the S-phase permits the chromosomes to spread irregularly through the cell. The second popular aberration type was stickiness, which created from partial dissolution of nucleoproteins and DNA de-polymerization. Akaneme and Amaefule[41] have proved that stickiness could be led to cell death because it results from the highly toxic and irreversible effect.

The chromosomal bridge was formed by breakage and reunion of sticky chromosomes. Low percentages of Laggards were also noted. Akaneme and Amaefule[41] have stated that Laggards are an obvious trait in cytotoxicity/genotoxicity types in research with extracts of medicinal plants. Moreover, Laggards are an origin of aneuploidy that they showed no ability to bind by spindle fibres. Laggard chromosomes do not take part in the normal division and the resulted daughter cells may have genetic dis-equilibriums.

Chromosome fragmentation was rarely observed in this study. Asthana and Kumar[42] recorded that formation of fragments in *V. odorata* extract may be caused by breakage in the terminal ends of chromosome or disability of chromosome thread to reunite and unfinished or disrepair of DNA.

A high percentage of Micronucleus was found in untreated infested seeds compared to *F. vesiculosus* and *S. platensis* treated infested ones. The loss of genetic materials indicated micronucleus formation. Hamedo and Abdelmigid[43] defined micronucleus analysis as the quickest, economical and efficient way of determining the genotoxicity of different chemicals. Celik and Aslanturk[44] confirmed that cellular death is a result of micronuclei (MN) due to the deletion of primary genes. Therefore, chemicals that cause harm to plant chromosomes could be dangerous also for mammalian chromosomes. This enhances the use of algae as safe biofertilizers, due to its effect on micronucleus in low values.

CONCLUSION

The examined two algae; *Fucus vesiculosus* and *Spirulina platensis* seemed to be good alternative bioinsecticides in controlling stored grain insect pests. These algae not only achieve high mortalities of different insect stages but also enable to activate germination and seedling development with low levels of abnormalities and without genotoxicity effects. This study recommends mentioned algae in protecting stored grains from attacking insect pests. Mixing seeds with algae powder could protect grains from insect attacking besides and improving plant growth rate.

SIGNIFICANCE STATEMENT

This study discovers the Bio-insecticidal effect of two types of algae against stored insect pests that can be beneficial for using alternate pest controlling method. This study will help the researcher to uncover the critical areas of applying pest control by algae that many researchers were not able to explore. Thus, a new theory on Bio-insecticides and understanding their safety effect on the environment may be arrived at.

ACKNOWLEDGMENT

We wish to express our appreciation to Dr. Doaa Mohammed Hammad researcher at Central Laboratory for Environmental Quality Monitoring, National Water Research, for helping and providing us with all the facilities in this study. We would like to immensely grateful to our colleagues and the researchers of the Department of Plant Protection, Faculty of Agriculture, Ain Shams University and staff members of the Department of Botany, Faculty of Women for Art, Science and Education for their continuous support and advise during this work.

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