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Efficiency of Three Bio-components against broad bean beetle (*Bruchidius incarnates)* and Their Effect on Germination, Seedling Growth and Cytogenetic Changes of *Vicia faba* L. plants.

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ABSTRACT

The effect of Radiant SC 12%, *Fucus vesiculosus* brown alga and *Spirulina platensis* cyanobacterium were evaluated on the mortality of adult *Bruchidius incarnates*. Data investigated significant differences where the percentage mortality by *F. vesiculolus* (0.25 and 0.50 gm) reached 100% after 14 days and 16 days respectively. Radiant (3.75 ppm) recorded 100 % mortality after 16 days while radiant (0.94 and 1.87 ppm) recorded 93.3% mortality after16 days. *S.platensis* (0.25gm and 0.50 gm) seem to be less potent against the insect where the percent mortality recorded 60 and 66.6 % after 16 days respectively. Results revealed a pronounced inhibitory effect on seed germination and seedling growth of *Vicia faba*. plants in infested seeds and radiant treatments; while fucus and spirulina showed a stimulatory effect. Cytogenetic analysis showed inhibition of mitotic activity with increased percentage of chromosomal aberrations in *Vicia faba*. plants in infested seeds and radiant treatments; both kinds of algae showed increasing of mitotic index and low percentage of abnormalities. The concentration 3.75 ppm of radiant exhibited the lower value of mitotic index (MI=6.7%) and the higher percentage of chromosomal abnormalities (CA=43.28%) compared to control (8.3% and 13.25%) respectively.

Keywords: bio-components, *Bruchidius incarnates, Vicia faba* L, germination.

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INTRODUCTION

It is well known that, of all demands of human, food is of prime importance however, post-harvest losses of seeds and grains as a result of infestations from storage pests are a serious problem. In this regard, attempts to protect stored grains (seeds) from insect infestation took place. Pesticides are chemical substances that are widely used against plant pests and diseases. The unwise usage of these synthetic insecticides may pollute the soil, air and irrigation canals, kill the natural predators, negatively affect man health and some may breakdown into toxic derivatives and causes phytotoxicity problems in sensitive crops due to the residual property, [1], Pesticides acted through a common mechanism of toxicity and conducted cumulative risk assessment. [2] Concluded that the insecticide Dichlorvos (DDVP) clearly poses a genotoxic riskand mutagenic effect on *Hordeum vulgare*seeds. A number of pesticides are used to protect agricultural products from diseases, weeds and insects, but residues of these chemicals lead to environmental pollution and pose threat to people and animals, [3] in addition to insect resistance to chemical or synthetic insecticides. Therefore, the search for new safer types of insecticides, less harmful and biodegradable to nontoxic products(e.g. those of plant origin), have recently attracted the attention of many scientists all over the world. Many Egyptian entomologists were interested in studying, the activity of extracts of many plants growing in Egypt against different insects among them was[4].So, most natural plant products do not have the problem of creating harmful residues or breakdown materials that would damage plants or harm human beings and animals. In fact, they hardly have any harmful effects on the plant-animal relationships in nature.

Broad Bean, (*Viciafaba* L.) is widely grown in Egypt forming the main source of protein. But, it was reported that broad bean is attacked by serious pests affecting both its quality and quantity. Broad bean *V. faba* is attacked by a number of insect pests which often cause extensive damages. Bruchid beetles (Coleoptera: Bruchidae) attack legume seeds and cause severe damage in the quality and quantity of the crop. They attack broad bean before or during harvest as well as in storage causing a serious damage. Broad beans are considered the most favored food for *Bruchidius incarnatus* (Boh) [5], where serious damage can be caused to stored dry beans on which this pest appear. The bio–agent, Radiant SC12% (Spinetoram) is a commercial reduced-risk pesticide that is naturally derived. The activity of spinosad is attributed to the metabolites spinosyns A and D, which are fermentation products of the soil actinomycete bacterium, [6] *Saccharopolyspora spinosa* has low mammalian toxicity and little toxicity to non-target insects and it degrades quickly when exposed to sunlight (UV light) .Spinosad has unique mode of action on the insect nervous system at the nicotinic acetylcholine receptors and it has additional effects on gamma aminobyturic acid or GABA receptor sites, leading to continuous activation of motor neurons and causing cessation of feeding, tremors of most muscles in the body and later, paralysis and death [7].

Spirulina platensis is a blue-green micro alga or cyanobacterium found in warm water and alkaline volcanic lakes.Spirulina has a soft cell wall made of complex sugars and proteins. Recent studies have demonstrated that in the microalga *Spirulina platensis* a blue protein called phycocyanin, belonging to the photosynthetic apparatus, has antioxidant and radical scavenging properties both *in vivo* and *in vitro* models [8]. [9] recorded that application of 5% concentration of water solution of the blue green alga, *Spirulina platensis*, can protect the host plants from being attacked by *Spodoptera littoralis* with the other means of integrated pest management. Furthermore, Cyanobacteria play an important role inmaintenance and build-up of soil fertility, consequently increasing rice growth and yield as a naturalbio-fertilizer [10].[11] owed the positive effect of N_2-fixing cyanobacteria on plant growth and yield of crops to the production of growth-promoting substances, i.e., gibberellins, cytokinins, auxins, abscisic acids, vitamins, antibiotics and amino acids. It also forms an important nitrogen balancing biological source of many crops.

Fucus vesiculosus, commonly known as: Bladderwrack, Sea kelp or Rock wrack, is a member of the Fucaceae family, a genus of marine brown algae found on rocky seashores of the temperate areas of the Atlantic and Pacific oceans [12]. [13] found that *Culex pipiens* mosquito larvae treated with brown algae were significantly affected and didn't reach the pupa stage due to the levels of protective polyphenolic compounds of brown algaeas important chemical defense significantly reducing palatability in response to direct mosquito attacks. Many species of marine algae including *Fucus vesiculosus* have been reported to be used as bio-fertilizers due to the presence of trace elements and in particular the growth hormone like substances such ascytokinins in them; they are used as foliar spray, application to soil, for soaking of seeds before sowing, enhancing the germination of seeds, increasing uptake of plant nutrients, giving resistance to frost and fungal

diseases, are effective for ripening of fruits, increasing shelf-life of the produce, improve the quality of the produce and serve as an excellent soil conditioner.

Mitotic index is not only a parameter of intensity of cell division, but also an indicator of cytotoxicity. According to [14], cytotoxicity is defined as a decrease in mitotic index and as an increase in the fraction of cells with c-mitosis, multi-polar anaphase, sticky chromosomes and laggards. A number of plants extracts have been reported to have antimitotic and chromosome damaging properties, [15]. The obtained results found by [16] revealed that dusting treatments of *Vicia faba* storing seeds with Black cumin and *Lupinus termis* powder showed statistically significant to highly significant depression in the mitotic index and affected the induction of highly significant percentages of abnormal mitosis. Genotoxicity is expressed as varying types of DNA damage and mutations, ranging from gene to structural or numerical chromosome changes. The presence of mitotic abnormalities and induction of micronuclei in interphase cells indicate genotoxic effect.

Therefore the present study aimed to evaluate the insecticidal efficiency of three different bio-components (Radiant SC12% (Spinetoram), *Spirulina platensis* cyanobacterium and the brown alga *Fucus vesiculosus*) against *Bruchidius incarnatus* adults as natural pesticides to control one of the most serious pests affecting broad bean, and their effect on germination, seedling growth and cytogenetic changes of *Vicia faba* plants.

MATERIALS AND METHODS

Culture of *Bruchidius incarnates:*

Stock of *Bruchidius incarnates* was obtained from infested bean bought from local market. Laboratory cultures of *Bruchidius incarnates* were maintained on uninfected bean grains *Vicia faba* L. (Var.Giza-716) obtained from Legume Research Department, Field Crops Institute, Agriculture Research Centre, Giza, Egypt. Adults of the insect were introduced into plastic jars containing bean grains. These plastic jars were covered with a muslin cloth to prevent insects escaping and to allow ventilation. Bean grains were kept in ambient laboratory conditions for the emergence of new *B. incarnatus* adults [17]. For all the experiments 1-7 days old, adult beetles were collected from cultures. All the experiments were kept aside at temperature 27±2°C and 65±5% relative humidity.

Laboratory bioassays:

Preparation of radiant solution:

A liquid formulation of Radiant 12% was obtained from Plant Protection Research Institute (Cairo, Egypt). Bio-insecticide was diluted in distilled water to make solutions of different concentrations for bean treatment. Different concentrations of the insecticide were prepared to test their effect on the adults of *B. incarnatus.* Three concentrations (0.94, 1.87 and 3.75 ppm) were prepared in which bean grains were dipped for 15 seconds; the treated grains were then left to dry under laboratory conditions. Each concentration consists of three replicates with 15 adults /replicate. For the control, adults were allowed to feed on untreated grains. Corrected mortality was calculated after 48 hours.

Source of algae used and method of treatment:

Spirulina platensis food supplement tablets obtained from DXN Company; the first Multi-Level Marketing (MLM) Company in Malaysia producing spirulina from the cultivation process to finished goods, where only the selected best species are naturally cultivated in a clean pond with no pesticides or herbicides applied, while dry *Fucus vesiculolus* brown algae were purchased from a local market in Cairo. Both *Spirulina platensis* tablets and dry *Fucus vesiculolus* was powdered by electrical grinder. Two amounts (0.25 and 0.50 gm) were used to study the effect of each alga on the adult of *B. incarnates*. Each amount was repeated three times, (15 adults/ replicate). Bean grains were treated with algae powder in shaker to harmony covering grains. Adults were allowed to feed on treated grains. Corrected mortality was calculated and recorded after 48 hours.

Seed germination and seedling growth:

Vicia faba grains of uniform color and size were thoroughly washed with distilled water and surface sterilized in 5% ethanol for 15 minutes and then washed with distilled water for several times. The first experiments were a laboratory bioassay. Ten seeds of each treatment (radiant and tow algae), infested seeds and control were placed on filter papers in 9 cm diameter Petri plates under normal laboratory conditions. Plates were incubated in a growth room at 25 ± 2^{o}C for 10 days. Each treatment was replicated three times. After 8 days of incubation, germination percentage of seeds was recorded [18], germination speed (GR), seed vigour index (VI), [19], relative germination ratio (RGR) and Percentage of germination Inhibition (I) [20] were calculated. The second experiment, which is actually a soil bioassay, was done in plastic potsby using the same above mentioned treatments for 10 days to study root, shoot and plant length.

Cytogenetic tests

Vicia faba seeds were placed in tap water for 48h to germinate at room temperature 25°C, The water was changed every 24 hrs. When the roots were 1-2cm long, cut, fixed in Carnoy's fixative chemical (1:3 acetic alcohol), hydrolyzed using 0.1N HCL, squashed and stained with Feulgen squash technique. Five temporary slides were prepared for each treatment and control; approximately 1000 cells per slide were examined. Mitotic index, phase index and mitotic inhibition were estimated. Chromosome abnormalities were scored in Pro-metaphase and ana-telophase. Slides were photographed using oil immersion (100 x) objectives and 15 x eyepiece Olympus microscope camera.

Micronucleus assay

The MN assay was conducted according to [21]. After the same method for mitosis, 1000 interphase cells per treatment from 5-7 separate slides were evaluated for micronuclei and expressed in terms MN/1000 cells. A treated /control (T/C) ratio was used to compare the efficiency of the MN scoring from the meristematic cells of *Vicia faba* L. root tips.

Statistical analyses

Means of percentage of adult mortality and all seed parameters was statistically analyzed using SAS program followed by Duncan-MSD test [22]. Statistical significant differences between individual means were determined by one way analysis of variance. Percentages of the mortalities were corrected according to Abbott's formula [23].

$$\% \text{ Corrected mortality} = (T-C) / (100-C)*100$$

Where: T: % mortality in treatment,
C: % mortality in check (control).

RESULTS AND DISCUSSION

Susceptibility of adult *Bruchidius incarnates* to three different bio-agents

Data in Table (1) and Figure (1), illustrated the effect of three bio-components (*S.platensis*, *F. vesiculosus* and Radiant SC12%) on the mortality of adult *B. incarnates*. Statistical analysis revealed highly significant differences between three components as shown in Table (1). Data investigated that the percentage mortality of *F. vesiculolus* (0.25and0.50 gm) reached 100% after 14 days and 16 days respectively, in the same line [13] found that when 2nd instar *Culex pipiens* mosquito larvae fed on dried marine algae, the larvae neither die nor develop normally; groups of larvae that received dried algae survived but they didn't go through the normal development in comparison with the control group, significantly different from the control ρ>0.0005; the study also showed that the brown algae *Padina pavonica* was the most effective species affect significantly the larvae growth rate as all mosquito larvae died at day 10 with ρ>0.0001,none of larvae treated with *Padina pavonica* reached fourth larval instars, significantly different from the control ρ>0.0005; larvae were significantly affected and didn't reach the pupa stage this may be due to brown algae contain high phenolic contents, our results are in agreement with those of [24] who reported that brown algae produce a specific

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type of tannin called phlorotannins; this larvicidal effect of brown algae may be due to phenolic, terpenoids or unsaturated fatty acids [25].

Table 1: Adult mortality (mean ± SD) of the Broad Bean Beetle, *Bruchidius incarnatus* treated with three different bio-agents

Exposure period		*S. platensis* (0.25 gm)	*S. platensis* (0.50 gm)	*F. vesiculolus* (0.25 gm)	*F. vesiculolus* (0.50 gm)	Radiant (0.94 ppm)	Radiant (1.87ppm)	Radiant (3.75ppm)	control	F. value	L.S.D
After 2 days	M. of dead adult ±sd	1± 0.5^{c}	1.5 ± 0.9^{c}	3 ± 0.3^{a}	5.5 ± 0.2^{a}	0d	1 ± 0.8^{c}	2 0.7^{b}	0^{d}	13.6	2.8
	Corr. Mortality %	**6.6**	**10**	**20**	**36.6**	**0**	**6.6**	**13.3**	**0**	-	-
After 4 days	M. of dead adult ±se	2 ± 1.4^{d}	2.5 ±0.2^{d}	6 ± 0.8^{a}	8 ± 0.3^{a}	3 ± 1.3^{c}	3.5 ± 0.4^{c}	4.5 ± 0.4^{b}	1 ± 0.2^{e}	25.6	3.1
	Corr. Mortality %	**13.3**	**15.5**	**39**	**52.3**	**19**	**22.3**	**29**	**5.6**	-	-
After 6 days	M. of dead adult ±sd	3.5 ± 0.4^{d}	4 ±0.4^{d}	8 ± 0.9^{b}	9.5 ± 1.7^{a}	5 ± 0.6^{c}	5 ± 0.4^{c}	8.5 ± 0.9^{a}	1 ± 0.2^{e}	54.2	1.9
	Corr. Mortality %	**23.2**	**26.2**	**53.3**	**63.3**	**33.3**	**33.3**	**56.6**	**5.6**	-	-
After 8 days	M. of dead adult ±sd	4 ± 0.5^{d}	4.5 ± 0.6^{d}	9.5 ± 1.2^{b}	11 ± 0.6^{a}	5.5 ± 0.87^{c}	6 ± 0.6^{c}	9 ± 0.6^{b}	1 ± 0.2^{e}	29.6	2.4
	Corr. Mortality %	**23.6**	**3.**	**63.3**	**73.3**	**36.6**	**40**	**60**	**5.6**	-	-
After 10 days	M. of dead adult ±sd	4 ± 0.5^{d}	5 ± 1.3^{d}	11.5 ± 0.5^{b}	12.5 ± 1.4^{a}	10.5 ± 0.4^{c}	11 ± 0.7^{c}	11.5 ± 1.6^{b}	2 ± 0.4^{e}	21.5	4.2
	Corr. Mortality %	**23.6**	**33.3**	**76.6**	**83.3**	**70**	**73.3**	**76.6**	**11.2**	-	-
After 12 days	M. of dead adult ±sd	5 ± 1.2^{c}	6 ± 0.2^{c}	12.5 ± 0.4^{b}	13 ± 0.9^{a}	12.5±0.4^{b}	13 ± 0.4^{a}	13 ± 0.6^{a}	2 ±0.4^{d}	32.3	2.1
	Corr. Mortality %	**33.3**	**40**	**83.3**	**86.6**	**83.3**	**86.6**	**86.6**	**11.2**	-	-
After 14 days	M. of dead adult ±sd	6.5 ± 0.3^{e}	7 ± 0.7^{e}	14 ± 0.21^{b}	15 ± 0.5^{a}	12.5 ± 1.8^{d}	13 ± 1.6^{d}	13.5 ± 0.1^{c}	2 ±0.4^{f}	14	1.6
	Corr. Mortality %	**43.2**	**46.6**	**93.3**	**100**	**83.3**	**86.6**	**90**	**11.2**	-	-
After 16days	M. of dead adult ±sd	9± 0.6^{c}	10 ± 0.9^{c}	15 ± 0.6^{a}	-	14 ±0.8^{b}	14 ± 0.3^{b}	15 ± 0.6^{a}	2 ± 0.4^{e}	33	1.6
	Corr. Mortality %	**60**	**66.6**	**100**	**-**	**93.3**	**93.3**	**100**	**11.2**	-	-

Means within a row for insect pest followed by different letters are significantly different (P < 0.05; using Duncan's multiple range clarifying by LSD test).

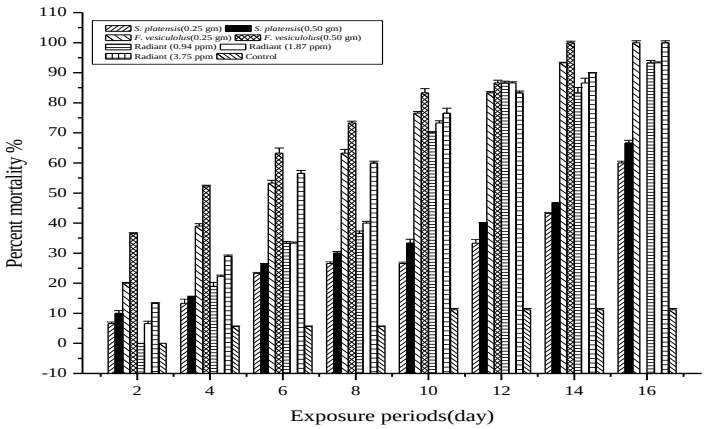

Fig (1): Adult mortality of *Bruchidius incarnatus* treated with three bio- agents

Radiant (3.75 ppm) recorded 100 % mortality after 16 days while radiant (0.94 and 1.87 ppm) recorded 93.3% after16 days. [26] tested six insecticides for their persistence and residual toxicity against first instar larvae of *H. armigera* on okra fruits, radiant was found to be most effective. Meanwhile, *S. platensis* (0.25gm and 0.50 gm) seem to be less potent against *Bruchidius incarnates* compared to the previously mentioned bio-agents, where the percent mortality recorded 60 and 66.6 % after 16 days respectively, in this aspect [9] found that cyanobacteria *Spirulina platensis* cell content showed no significant effect on *S. littoralis* larvae at concentrations of 0.5, 1.0, 2.5 mg cell content dry weight / L dipping solutions. However, significant effect was observed on both pupae and moths at these low concentrations. At 5%concentration larval mortality and malformation increased and 100% mortality was obtained.

Our results revealed that mortality of adult *B. incarnates* increased by increasing both radiant concentration and algae amounts (*S.platensis* and *F. vesiculosus)* [17].

Seed Germination and plant growth

The percentage of germination of *Vicia faba* seeds was decreased after insect infestation and radiant treatment as compared with the control. The highest radiant dose showed the lowest germination percentage, while the use of algae *Spirulina platensis* and *Fucus vesiculolus* induced high percentage of germination as well as control. The Germination Percentage (GP), Germination Speed (GR), Relative Germination Ratio (RGR) and Vigour index of seeds decreased with increasing concentration of radiant, while the percentage inhibition on germination (I)increased as the concentration increased.

Data in Table (2) showed that (GR) of *V. faba* seeds relative to control decreased significantly in infested seeds and with increasing concentration of radiant (3.75ppm) reaching 10 and 17.5 respectively, as well as Germination Percentage (GP), and Relative Germination Ratio (RGR) showed the same effect. On the other hand, algae showed a reversible effect.

Table 2: Seed germination and plant growth of *Vicia faba* (means ± S.D.) treated with radiant, algae and infested seeds.

Treatments	Seed germination					plant growth		
	GP	GR	RGR	I	Vigour index	Radical length	Shoot length	Plant length
Control	93±1^{a}	23.25±0. 25^{a}	1±0. 0^{a}	0±0^{d}	86.65±1^{a}	2.40±0. 4^{ab}	3.80±0. 4^{b}	6.20±0. 8^{bc}
Infested seeds	40±2^{d}	10.00±0. 5^{d}	0.43±0. 025^{d}	56.98±2.15^{a}	35.25±2^{d}	1.25±0. 25^{bc}	3.50±0. 5^{b}	4.75±0. 75^{cd}
Radiant 0.94	90±3^{ab}	22.5±0. 75^{ab}	0.96±0. 035^{ab}	3.22±0.035^{cd}	84.42±3^{ab}	1.87±0. 87^{abc}	3.71±0. 29^{b}	5.58±0. 58^{bcd}
Radiant 1.87	85±2^{b}	21.25±0. 5^{b}	0.91±0. 02^{b}	8.60±0. 02^{c}	79.80±2^{b}	1.50±0. 5^{abc}	3.70±0. 2^{b}	5.20±0. 7^{bcd}
Radiant 3.75	70±2^{c}	17.50±0. 5^{c}	0.75±0. 02^{c}	24.73±0. 02^{b}	65.74±2^{c}	1.04±0.16^{c}	3.22±0. 22^{b}	4.26±0. 38^{d}
fucus	93±2^{a}	23.25±0. 5^{a}	1.00±0. 025^{a}	0±0. 025^{d}	84.78±2^{ab}	2.59±0. 09^{a}	5.63±0. 37^{a}	8.22±0. 28^{a}
spirulina	92±1^{a}	23.00±0. 25^{a}	0.98±0. 015^{a}	1.07±0. 015^{d}	85.56±1^{a}	2.55±0. 55^{ab}	4.04±0. 04^{b}	6.59±0. 59^{ab}
F value	298.8	298.7	264	340	274	5.52	18	14.16
LSD.	5.4	1.3	0.06	5.4	5.4	1.3	0.89	1.69

Means within a column for different treatments and concentrations followed by different letters are significantly different ($P < 0.05$; using Duncan's multiple range clarifying by LSD test).

The inhibition of seed germination by insect might be explained by the fact that the insect could act as a seed predator larval feeding and effectively kills the embryo or removes so much endosperm that the seed cannot germinate [27]. [28] found that, infestation by multiple larvae reduced the germination frequency of catclaw acacia (*Acacia greggii*) and blue paloverde (*Parkinsonia florida*).

The effects of radiant and other bio-insecticides on germination of plants were explained by several authors; among them were [29] who stated that, the crude hydroalcoholic extract obtained from *A. indica* leaves (biopesticide) exhibits inhibitory effects on *P. vulgaris* seed germination and radicle growth, these effects increase with extract concentration (w/v). [30] found that, as concentration of Neem extract increased except in 5%, the vigour index and tolerance index of *Vigna radiate*were found to be significantly decreased, when compared to control.

The effect of algae on *V. faba* seeds could be explained by several authors; [31] determined that a number of cyanobacteria produce, accumulate and liberate 3indol acetic acid; [32] recorded the activity of auxin and cytokinin by three cyanobacterial strains. [33] reported that, cyanobacteria produces bioactive compounds including plant growth regulators such as naphthalene acetic acid (NAA). All these compounds have the ability to stimulate germination and growth of plants.

Table (2), indicates that there is significant differences among root, shoot and plant lengths respectively ($p<0.01$). Results indicate that root, shoot and plant lengths decreased in infested seeds and by increasing radiant concentration when compared with control, while plant length increased after the effect of both algae types relative to control. The root length of *V. faba* reached 1.04 cm and 1.25 cm after 3.75ppm radiant treatment and insect infestation respectively compared with 2.4cm of control, while shoot length reached 3.22cm and 3.5cm after 3.75 ppm radiant treatment and insect infestation respectively compared with 3.8cm of control. On the other hand, the root length of *Vicia faba* reached 2.59cm and 2.55cm after the use of fucus and spirulina respectively, whereas the shoot lengths were 5.63cm and 4.04cm after using fucus and spirulina respectively where they gave the highest plant heights compared to control.

Concerning the effect of the insect on plant growth, seed beetle infestation caused depletion of cotyledon reserves that may slow plant growth and hence reduce the probability of establishment [28].

The data of radiant doses were similar to those caused by Neem bio-insecticide [29] showed that neem extract not only inhibits seed germination, but it also interferes with post-emergence by influencing plantule growth and reducing radicle length. The decrease in plumule length can cause by inhibiting in cell division and elongation and or decreasing in hormones such as acetic acid and gibberellin. The inhibition of shoot and root elongation is a result of reduction in the cell division due to damage of cell membrane caused by allelochemicals, [30].

Algae extraction effects on seedlings may refer to that, algae contain the nutrient elements and growth hormones that increased seedling growth. More overproduction of bioactive molecules such as auxins, production of secondary metabolites linked to biocontrol of bacterial and fungal diseases as well as improving soil structure and porosity through secretion of polysaccharides aiding in soil aggregation are the most important functions of cyanobacteria or blue-green algae in soil [34]. [35] stated that algal extract (0.1%) can be recommended in agriculture to increase quality of bean plants and when are used for organic farming, can reduce our dependence on chemical fertilizers. [36] investigated the impact of algae extract application to thirty of 12 year old Anna Apple trees, the results showed that the applied of algae extract was very effective in stimulating the shoot length, leaf area, total leaf carbohydrates and leaf mineral content.

Cytogenetically studies

Mitotic Index and Phase Index

The cytological observation revealed that the mitotic index (MI) decreases in the infested seeds and after radiant treatment with concentration sincreasing from 0.94ppm to 3.75ppm in the test plant (Fig.**2**) relative to control. The mitotic index was inhibited to 6.7% after 3.75ppmradiant dose which was significantly lower in relation to control (8.3%). On the other hand, the mitotic index increased after using fucus and spirulina with respect to control, reached 12.1% and 9.2% respectively.

The percentage of dividing cells in prophase, metaphase and ana-telophase was demonstrated in (Fig.**4**). Prophase stage after insect infestation and radiant treatment had highest mean number of cells; this is followed by metaphase and ana-telophase in the same order. On the other hand, algae treatments showed the highest number of cells in prophase stage.

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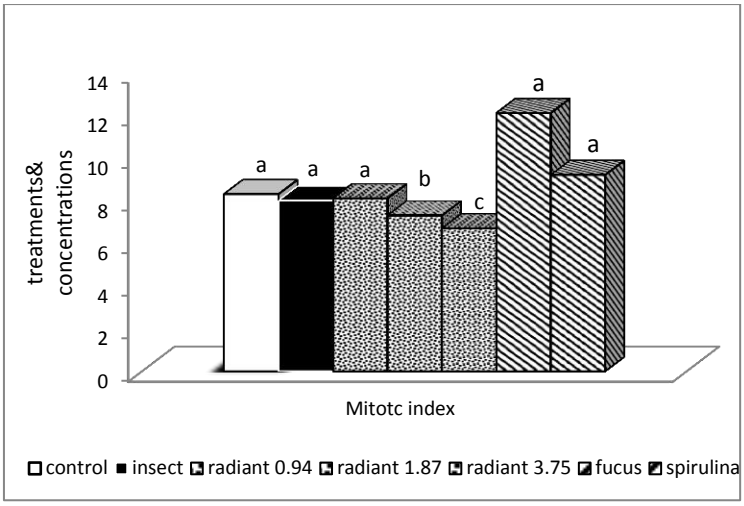
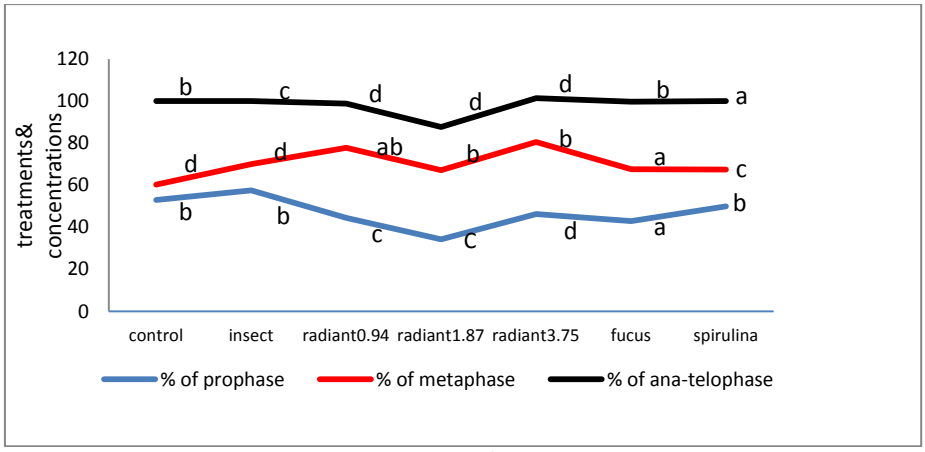


Column with different letters are significantly different (P < 0.05; using Duncan's multiple range clarifying by LSD test), where F value= 323, LSD. = 8.3

Fig 2: Cytotoxic effect of *Vicia faba* root tip cells scored in cells treated with radiant, algae and infested seeds



Column with different letters are significantly different (P < 0.05; using Duncan's multiple range clarifying by LSD test), where Prophase F value= 88.8,LSD= 4.5. Metaphase F value= 91.5, LSD= 4.17. Ana-telophase F value= 54.93, LSD = 5.8.

Fig 4: Frequency of mitotic phases in *Vicia faba* root tips treated with radiant, algae and infested seeds

Generally infested seeds and radiant concentrations showed significant inhibitory effect on mitotic activity, even at lowest radiant concentration. MI inhibition could be due to inhibition of DNA synthesis or a blocking in the G2 phase of the cell cycle, preventing cell from entering mitosis. According to [37], the reason of inhibition of cell cycle is the damage of chromosome areas containing special proteins by the pesticides. This inhibition caused by the lack of DNA polymerase, the lack of enzymes and proteins which are required for spindle apparatus to work properly could be the direct reason for the inhibition of cell cycle,[38].

Our results suggested that radiant possesses inhibitory mito-depressive effects that might prevent DNA synthesis. The reduction in number of dividing cells in roots produced by the cytotoxic effects of compounds found in radiant bio-agent was strongest in root tip cells. Accordingly, it may be concluded that radiant has a complex mixture of compounds that may possess cytotoxicand mutagenic properties.

The enhancement of mitosis caused by algae treatment could be explained by several studies using algae as bio-fertilizer, among them was [39]. Algae bio-fertilizers provide some growth regulators like auxin, abscisic acid, gibberellins and vitamin B12 which are very necessary for plant growth and development, [40].Results of the present study indicate that fucus and spirulina have the potential as effective and safe bio-fertilizers enhancing *Vicia faba* growth. It was reported that exogenous gibberellin and cytokinin, pretreatments stimulated cell division in normal conditions, stimulating activity of mitosis was previously reportedafter the use of bio-fertilizers by stimulating the plant defense response, [41]. On the other hand,

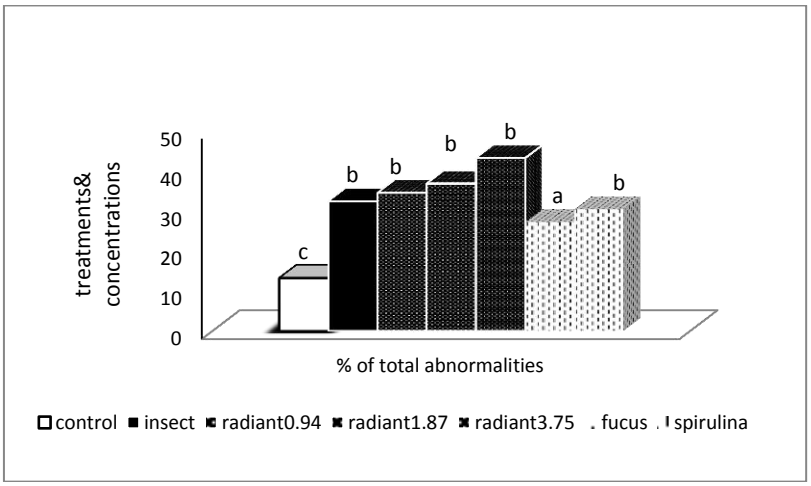
progression in cell cycle is regulated at two points, the G1/S and G2 /M;cytokinins were found to activate cell division through induction of D-type of cyclins mediators of internal and environmental stimuli to drive cell division, [42]. However, the increase in cell division was found to be related to the length of telomeres, the nucleoprotein structure protecting the end of chromosomes, and its activity promote malty entry into cell cycle division, [43].

The phase index results occurred by [44] after the use of *Azadirachta indica* A. Jussextract on *Allium cepa*L. were the same as in radiant treatments and infested seeds, this may be attributed to that the spindle is so disturbed, reduced and may eventually disappear, resulting in the blocking of cell division at the end of the prophase stage or even metaphase, . Thus, it can be stated that the blockage of cell divisions was a result of the "freezing" of cells in the G2/M phase of the cell cycle; this was accompanied by an accumulation of prophases with very condensed chromosomes [45].

It can also be deduced that prophase build up is the result of prevention or delay of spindle fiber formation. The highly significant increase in mitotic indices after algae treatment was concomitant with the large amount of cell present in prophase stage recording significant values. These increased number of prophase cells were the ninfiltrated by the "metaphase-checkpoint "and oranaphase promoting complex, leading to the low percentage of cells present in metaphase and normal percentage of cells in telophase compared to their respective control. Adaptation in distribution of dividing phases, followed by stimulation in mitotic index was previously noted by [46].

Abnormalities

Microscopic examination of squashed *Vicia faba* root tip cells showed that infested seeds and radiant treatment induced a number of mitotic abnormalities when compared with the respective control. The mitotic abnormalities of *V. faba* meristematic cells increased with increasing concentrations reaching its highest value (43.28%, 32.5%) after 3.75ppm radiant concentration and insect infestation as compared with control (13.25%), (Fig.**3**), while fucus and spirulina showed lower percentage of aberrations.



Column with different letters are significantly different (P < 0.05; using Duncan's multiple range clarifying by LSD test), where F value= 83.9,LSD. = 3.4

Fig 3: Genotoxic effect of *Vicia faba* root tip cells scored in cells treated with radiant, algae and infested seeds

The most common chromosomal abnormalities induced by all treatments were: irregular prophase, spindle disturbance, stickiness, bridges, laggards, fragments and micronuclei, Table (**3**). However, these different types of abnormalities after algae treatment were recorded in low percentages and no fragments were recorded in both types; in this aspect, [35] mentioned that algae extract as a new bio-fertilizer containing N, P, K, Ca, Mg, and S as well as Zn, Fe, Mn, Cu, Mo, and Co, some growth regulators, polyamines, natural enzymes carbohydrates, proteins and vitamins applied to improve vegetative growth and yield. [47] reported that chromosomal aberrations that resulted from different treatments indicate a clastogenic effect of the tested materials. The abnormality observations may be due to the nucleotoxic action of the extract or the

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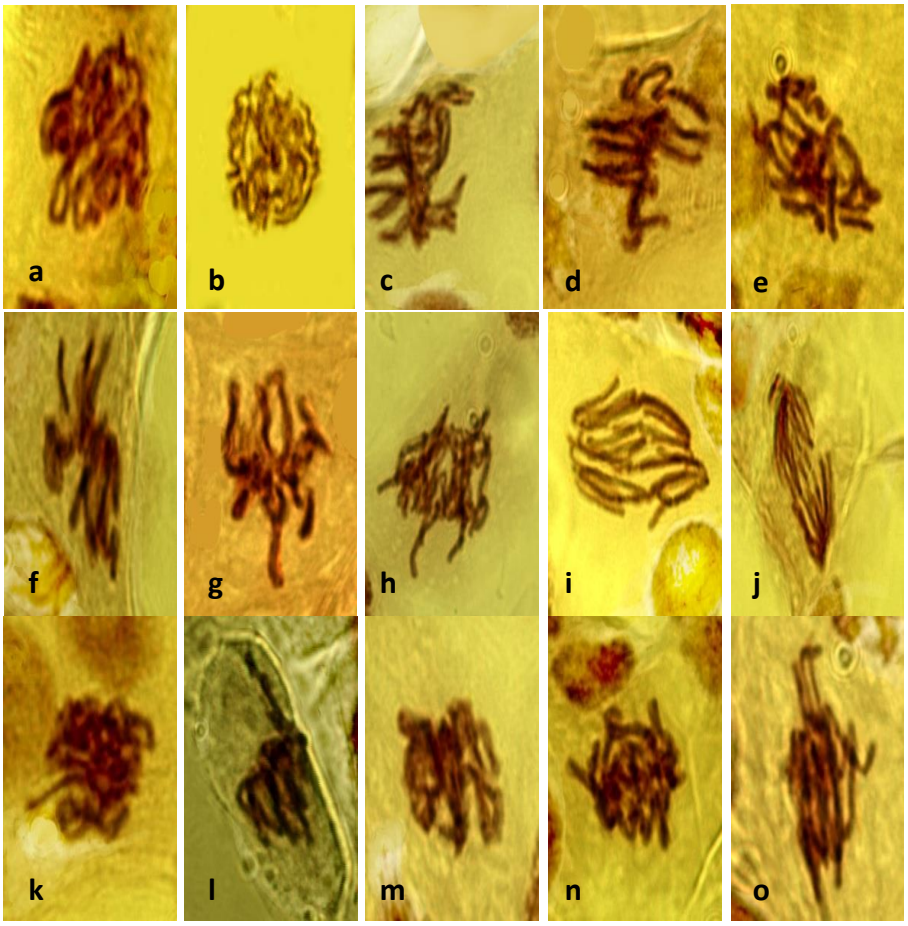
disturbance of the formation of spindle fibres during cell division which leads to chromosomal aberrations, as reported by [48]. So, the chromosomal aberrations in this study indicate that radiant treatments can lead to mutation or can act as clastogene.

Table 3: Percentage of different types of abnormalities in *Vicia faba* roots (means ±SD) treated with radiant, algae and infested seeds

Treatments & Concentrations (%)	Types of abnormalities					
	Disturb.	Irr. prophase	Stick.	Bridge	Lagg.	Frag.
Control	63.63	18.18	9.09	9.09	0	0
Insect	42.30	23.07	15.38	15.38	3.84	0
Radiant 0.94	46.42	14.28	17.28	7.14	7.14	7.14
Radiant 1.87	40.74	18.51	18.51	11.11	3.70	7.40
Radiant 3.75	37.93	24.13	20.68	6.89	6.89	3.44
Fucus	66.66	12.12	9.09	9.09	3.03	0
Spirulina	60.71	3.57	17.85	17.85	0	0

Disturbance was the most common abnormality and may be caused by inhibition of DNA synthesis at S-phase of cell cycle [49], or due to disturbance of spindle apparatus which allows the chromosomes to spread irregularly over the cell [50], (Fig. 7,plates: c - i).



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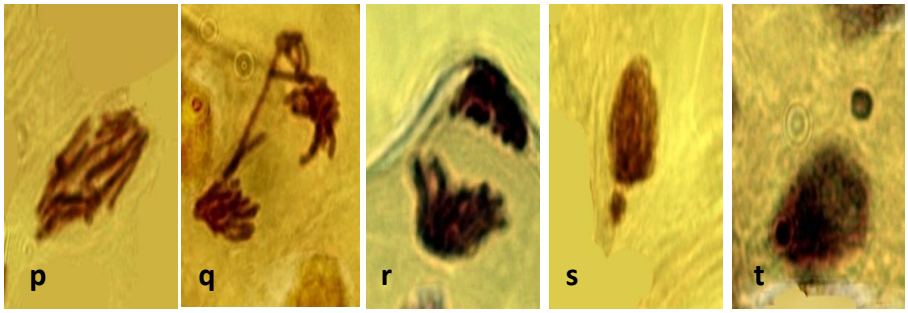

Fig.7: Different types of chromosome aberrations of *Vicia faba* L. root meristems induced byinsect, radiant and algae,(a, b): irregular prophase, (c):disturbed metaphase, (d, e): disturbed metaphasewith laggards, (f, g): disturbed metaphase with a fragment, (h): disturbed preanaphase, (i): disturbed anaphase with multibridge, (j): anaphase with multibridge and a fragment, (k): stickiness in prophase, (l, m, n): stickymetaphase and disturbed, (o): sticky and disturbed anaphase with multibridge, (p): sticky anphase withmultibridge, (q): sticky and disturbed anaphase with single bridge, (r): severe stickiness in anaphase,(s, t): interphase with macronucleus . Magnification is 1000X.

Chromosome stickiness was one of the common chromosomal aberrations and was recorded in a considerable percentage. [48], stated that stickiness might be due to DNA depolymerization and partial dissolution of nucleoproteins, breakage and exchanges of the basic folded units of chromatids and the stripling of the protein covering of DNA in chromosomes. There is an agreement that stickiness reflects highly toxic and irreversible effect that probably leads to cell death [51], (Fig.7,plates: k - r).

Another remarkable abnormality was chromosomal bridge. The bridges in the cells were probably formed by breakage and fusion of chromatids or sub-chromatids, [52]. On the other hand, bridge formation could be caused by chromosomal stickiness or chromosomal breakage and reunion, [49]. (Fig.7,plates:i, j, o, p, q, r).

The laggards were demonstrated in the present study, the lagging chromosome was possibly formed due to the inhibition of centromeric and spindle activity which inhibits chromosome movement to either of the poles and due to the interaction of drug with protein of the spindle apparatus,[53]. Laggards have been found as a regular feature of many cytotoxicity/genotoxicity studies with medicinal plant extracts [44]. Furthermore, Laggards are a potential source of aneuploidy because they lost the ability to attach by spindle fibers; they do not participate to the normal division and cause genetic dis-equilibriums between daughter cells [54]. (Fig.7,Plates: d, e).

The fragments (Fig.7, Plates: f, g, j) were noticed in the present study either due to terminal breaks in the chromosome or failure of chromosome thread to rejoin, [53]. Fragmented metaphase caused by extract of *A. vulgaris* is generally considered due to unfinished or disrepair of DNA, [55].

Mitosis anomalies such as bridge, laggards, breakage and micronuclei result from clastogenic effects on nucleus chromosomes, [38].The presence of these types indicated the clastogenic effects of radiant on *Vicia faba*.

Micronucleus assay

The induction of micronucleus formation was generally observed in all tested concentrations of radiant bio-agentand significantly when compared with the negative control ($p < 0.05$), the values were lower in F1 cells than in meristematic cells, (Fig. 6).

An *in vitro* MN assay can detect both clastogens and aneugens as well as mitotic delay, apoptosis, chromosome breakage, chromosome loss and non-disjunction, (Fig. 7,Plates:s, t). Micronucleus formation implies loss of genetic materials. Moreover, micronucleus analysis is considered to be one of the most economical, quickest and most effective ways in determining genotoxicity of different chemicals [56].Micronucleus (MN) refers to the fragment of damaged chromosomes or whole chromosomes which fail to find their way onto the spindle during cell division [57]. Micronuclei (MN) can cause cellular death due to the deletion of primary genes [58]. With reference to the significance of micronuclei test in plants for human

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health hazards it is recognized that if a chemical compound causes damage to chromosomes in plant cells, it can be potentially dangerous also for the chromosomes of mammalian cells.

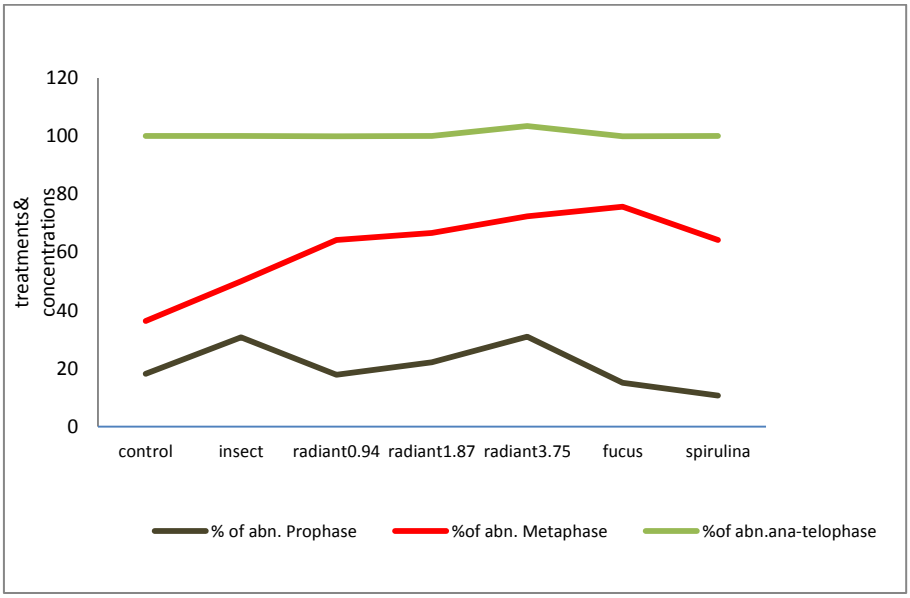
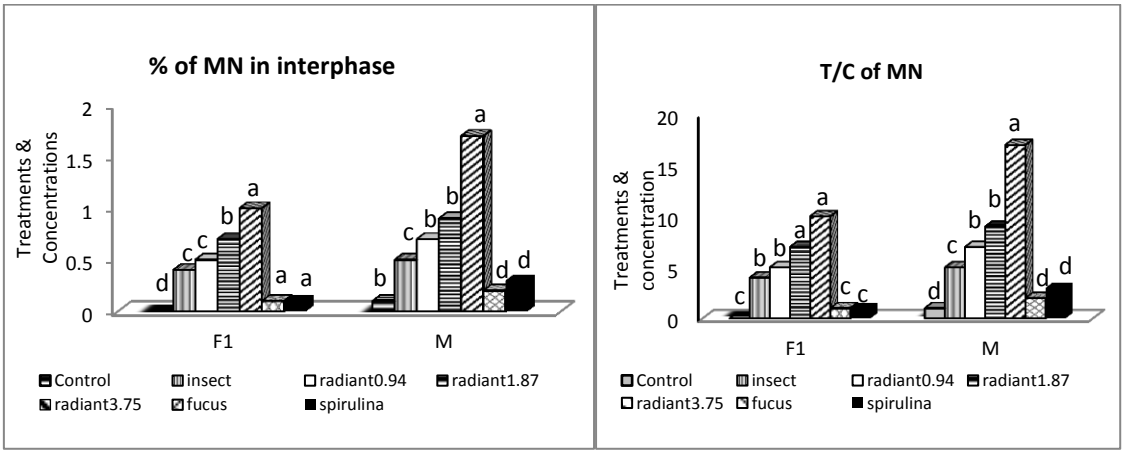


Fig 5: Percentage of abnormalities of mitotic phases in *Vicia faba* root tips treated with radiant, algae and infested seeds



Column with different letters are significantly different (P < 0.05; using Duncan's multiple range clarifying by LSD test), where% of MN in interphase (F1) F value= 27.2, LSD= 0.3 - % of MN in interphase (M) F value= 101.3, LSD= 0.2- T/C of MN (F1)) F value= 242, LSD= 1.6 - T/C of MN (M)) F value= 186.2, LSD= 0.8

Fig 6: Micronucleus assay of *Vicia faba* root tip cells scored in F1 and meristem M cells treated with radiant, algae and infested seeds

CONCLUSION

All results, point to the fact that radiant has the features of being a potential mutagen that has the capability of inducing damage in plant genetic material including some clastogenic changes; while examined algae are considered to be a safe biological control agent in addition to its ability in stimulating germination and seedling growth with no genotoxic effect. Therefore both fucus and spirulina can act as desirable biological fertilizers for organic farming instead of chemical fertilizers. Algae used are nontoxic, harmless and effective for attaining better germination, growth and yield; so it is important to take necessary steps to cultivate them on a large scale. The present study also recommended using examined algae in preserving stored grains to reduce damage caused by stored insect pests. It will be also beneficial to mix seeds with algae before planting to protect plants from insect infestation from one side and improving plant growth from the other side.

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