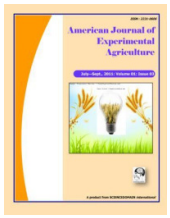

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Uses of Jellyfish in Pre Sowing Seeds Treatment and Pest Control

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Authors' contributions

This work was carried out in collaboration between all authors. Author OSH brought and prepared jellyfish, designed the study and wrote the protocol. All authors managed the analyses of the study, performed the statistical analysis. Author OIS wrote the first draft of the manuscript. Author RMS designed the experimental procedure and wrote the part of the pest control of the study. All authors read and approved the final manuscript.

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ABSTRACT

Aim: Preliminary experiment on the effect of liquefied Jellyfish on seedling growth and the digested solution of jellyfish as insecticides.
Place and Duration of Study: Natural Products Research Department, National Center for Radiation Research and Technology, Atomic energy authority.
Methodology: Seeds of anise, canola, coriander, cumin and dill were experimented to study the effect of presowing soaking treatment in liquefied Jellyfish on seedling growth. Also, different concentrations of digested solution of jellyfish were examined as bio-insecticide against two insects *Oryzaephilus surinamensis* L. and *Bactrocera zonata* (Saunders).
Results: Enhancement in germination parameters were observed in comparison to untreated control. The changes in protein pattern in seedlings from seeds soaked in liquefied jellyfish solution were investigated. Most of electrophoretic protein patterns in coriander weren't affected by soaking in jellyfish. But, in dill or in canola seedlings new bands were created in samples from seeds soaked in jellyfish compared to those separated from control soaked in water. On the other hand, the digested solution of jellyfish was highly toxic with LC_{50} of 2.06 and 1.14 % for *O. surinamensis*

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and *B. zonata*, respectively.
Conclusion: These data suggest that liquefied jellyfish is useful as a priming solution for plants and its digested solution as bio-insecticide.

Keywords: Jellyfish; electrophoresis; soaking; bio-insecticide.

1. INTRODUCTION

Jellyfish, *Cyanea capillata* represents a problem for many vacationers on Mediterranean Sea shore as it stings them. It contains important elements that can be used in fertilization, foliar spraying or other uses in scientific research [1]. Jellyfish was examined as bio fertilizer [2], [3]. Also, an application method for producing liquid fertilizer from whole jellyfish in Kobe University was carried out [4].

Anise (*Pimpinella anisum* L.), is an annual important spice and medicinal plant belonging to the family of *Apiaceae*, and native to Mediterranean region. Today, anise seeds are an important natural raw material which is used for pharmaceutics, perfumery, food and cosmetic industries [5]. Recently, this spice plant has drawn attention of consumers due to the antimicrobial, antifungal, insecticidal, and ant oxidative effect of this herb on human health [6,7]. Canola, *Brassica napus* L. seed contains over 40% oil [8]. After the oil has been removed from the seed, the protein-rich meal that is left behind is generally used as a protein source in livestock and aquaculture industries [9]. The growing demand for canola oil worldwide implies that more meal will be produced as a result of the increased oil extraction. Coriander, *Coriandrum sativum* L. is a hardy annual plant. The term cilantro refers to its leaf tissue, while the dry fruits are known as coriander mericarps [10]. The use of plants as medicines began during the earliest years of human evolution. *Nigella sativa* commonly known as the black cumin seed is an annual herb that belongs to the botanical family of *Ranuculaceae* [11]. It has been employed for thousands of years as spice and food preservative, as well as a protective and curative remedy for numerous disorders. It is known to have many medicinal properties in traditional medicine [12]. Dill weed is the member of the *Umbelliferae* family, a large group of flowering herbs and spices. It is a unique perennial herbal plant in the sense that both its leaves as well as seeds are used as a seasoning. It is considered to be one of the most important medicinal plants.
Priming is a physiological method that improves seed performance and provides faster, synchronized germination [13]. Seed priming has been reported to be better strategy in overcoming micronutrient deficiencies, enhance the speed of germination [14] and increase yield [15].

Insect control relies heavily on the use of organophosphates, carbamates, pyrethroids, and other classes of insecticides. However, significant insect resistance has emerged. In addition, residual agrochemicals in the environment have been causing a serious impact. Natural products are well known to have a range of useful biological properties against insect pests [16]. Some alternative source of natural origin for applying ecologically viable pest control strategies were reported [17]. Animals are a source of extracts providing therapeutic activity from which several organic substances have been isolated and some of them are currently applied as compounds of biomedical interest such as drugs and insecticides. The biotoxins like jellyfish venom included among these compounds [18]. Moreover, insecticidal activity of digested marine invertebrates was never studied before. Hence, in this study, we used different pest species to survey the ability of using jellyfish as a natural insecticide.

Bactrocera zonata (Sauners) considered one of the most important fruit pests in several parts of the world. It attacks a large host range of fruit and vegetables hosts; such as mango, peach, fig, guava, citrus, tomato and apple, [19]. *Oryzaephilus surinamensis* L. is the most common specie attacking stored grain and other products.

The purpose of this work is to study the effect of presowing in liquefied jellyfish on plant growth of anise, canola, coriander, cumin, and dill. Also, protein electrophoresis was carried out in some seedlings grew from primed seeds. In addition, the efficacy of digested jellyfish solution in controlling *Oryzaephilus surinamensis* and *Bactrocera zonata* were assessed.

2. MATERIALS AND METHODS

2.1 Jellyfish Preparation

Jellyfish obtained at the Mediterranean Sea shore, Alexandria, Egypt. The outer layer of Jellyfish weight (3-4 kg) were liquefied in an oven and preserved in refrigerator. A part of liquefied Jellyfish was used as priming solution before planting. Other part was digested in pure sulfuric acid and perchloric acid to dryness then diluted with distilled water for pest control experiment.

2.2 Seeds Germination

Preliminary experiments on seeds priming in liquefied solution of jellyfish in the field of agriculture were carried out to test their ability for germination. The seeds of anise, canola, coriander, cumin, and dill soaked in liquefied jellyfish solution for 30 minutes but anise seeds soaked for 16 hours, 20 seeds planted for each in little pots. Germinated seeds were counted for two weeks; the seeds considered germinated just protrusion of radical. Each treatment was replicated five times. Shoot and root length (cm^{-1}) were measured from culms base to the tip of the longest leaf or root.

2.3 Protein Electrophoresis

Protein pattern of liquefied jellyfish, canola, coriander and dill seedlings were assessed [20] based on a defined methanol-chloroform-water mixture for the quantitative precipitation of soluble as well as hydrophobic proteins from dilute solutions. The effectiveness of this method is not affected by the presence of detergents, lipids, salt, buffers, and *β*-mercaptoethanol.

Gel staining it is preferred to stain the gels by Comassie blue stain (Bio-Safe) from Bio-Rad, Cat# 161-0786. Chloroform-Methanol Precipitation, Concentration and detergent removal method used according to [21].

2.4 Effect of Digested Jellyfish Solution against Insects

2.4.1 Insects used

Bactrocera zonata (Saunders): Laboratory strain of *B. zonata* was obtained from the National Research Center, Giza, Egypt. Adults were fed on a mixture of sucrose and protein The larvae reared on artificial media at 25±2℃ and 60±5% R.H [22] to be used in the present experiments (3^{rd} instars larvae).

Oryzaephilus surinamensis L.*: O. surinamensis* used in all experiments were derived from a laboratory culture initially established from adults collected from infested wheat. Throughout the experiments, insect cultures were maintained under controlled laboratory conditions feed on the wheat grains and flour (10:1) with 5% Brower's yeast at 28-30℃ and 70±5% RH and continuous darkness. The newly emerged adults (2 days) were used in this study.

Test Procedures: the different concentration (0.5%, 1%, 1.5%, 2%, 2.5%, 3% and 3.5%) were prepared from stock of liquefied jellyfish solution by diluting with water. 1ml of each concentration was applied to filter paper placed in a Petri dishes (7cm). Ten of the tested insects were placed in the Petri dishes. All treatments were replicated 3 times. The percentages of mortality were recorded daily for 6 days. Data were corrected to control mortality using Abbott's formula [23]. Values of LC_{50} were calculated according to the method of Finney [24].

2.5 Statistical Analysis

Data obtained were statistically analyzed by using Costat statistical program software, 1990 and Duncan's multiple range test [25] at 5% probability level to compare the differences among time means.

3. RESULTS AND DISCUSSION

3.1 Seeds Germination

Enhancement was noticed in the germination acts in shoot and root lengths obtained from seeds soaked in jellyfish as compared to untreated ones that were soaked in water only (Table 1). Also, the data showed that dill seeds had the best growth in shoot, root and plant lengths as affected by soaking in jellyfish. On the other hand, coriander, followed by anise, cumin and canola seeds had good germination compared to control, but lower than dill seeds germination as illustrated in the same table.

Jellyfish comprises several useful elements and the heavy metals present in it mainly not detected. In addition to the presence of nitrogen that plays an important role in plant nutrition [1]. The insignificant change in parts or whole

seedlings of anis was observed and this may be attributed to length of soaking period. But cumin show significant change in parts or whole seedlings produced from seed soaked for 30 minutes. Seeds priming (seeds enriched with micronutrients) could increase the growth and yield, this in agreement with the results obtained on cumin [26] and on dill [27]. Seeds soaking in stock Jellyfish influenced seedlings growth, so it may be considered as growth promoter. But to support the result obtained need to apply on large scale.

3.2 Protein Electrophoresis

Protein bands of lequified jellyfish solution was represented in Table 2, six bands were separated and ranged between 282 - 38 kD. It had the most bands of marker protein but different in its Rf and MW. Brinkman [28] determined protein of box jellyfish by SDS-PAGE, a multiple sequence alignment of the five protein sequence, several short, but highly conserved regions of amino acids coincided with a predicted trans membrane spanning region, which could be involved in a pore-forming mechanism of action. Furthermore, remote protein homology predictions for the family of box jellyfish toxins suggested weak structural similarities and, hence, inferred function to pore-forming insecticidal 6-endotoxin proteins. Ruan, et al. [29] reported that jellyfish, *Cyanea capillata* contained 247 amino acids.

Table 3 and Figs. (1 and 2) referred that band no. 1 was found in canola seeds soaked in jellyfish and disappeared in control, as well as in protein band no. 4. On the other hand, band no. 3 clearly appeared in canola sample from control seedlings and disappeared in sample soaked in jellyfish. In case of band no. 2 was found in both samples, but the sample soaked in jellyfish had a bigger molecular weight than that in canola control sample. New protein bands were created in both samples as a result of treatment.

Canola protein is stable at most pH values with no significant ($P > 0.05$) changes in droplet size during storage for up to 7 days at room temperature [30]. Also, the protein molecular structure in endosperm tissues in newly developed black and yellow-type canola seeds by using synchrotron-based Fourier transform infrared micro spectroscopy were characterized [31]. The results showed that both the yellow and the black-seeded canola contain the same proteins but in different ratios.

The emergence of new proteins with small molecular weight when seeds soaked in liquefied jellyfish is probably due to the effect of transformation of iron and sulphur ions found in jellyfish to other forms led to similar effect to free radical effective, but to a lower degree, resulting in the degradation of the peptide bonds and rearrangement of protein molecules, finally an accumulation, with some composed of new proteins (short chain proteins).

Table 4 and Figs. (1 and 2) showed that bands no. 1 and 2 of coriander protein sample had the same Rf and MW as in sample primed in jellyfish. Meanwhile, band no. 3 had newly appeared in coriander protein pattern being affected by soaking in jellyfish. Zhuang et al. [32] supported the feasibility of jellyfish gelatin as a natural antioxidant, polypeptide provider. They added that, enzymatic hydrolysis and ultra filtration could be potent future processing technologies to utilize the abundant jellyfish resource.

Table 5 and Figs. (1 and 2) illustrated that the differences were found between dill proteins of control and soaked in jellyfish. Band no. 1 appeared in sample soaked in jellyfish and disappeared in control sample. The opposite happened in band no. 2 it was found in control sample and disappeared in dill sample soaked in jellyfish. Oshchepkoval et al. [33] isolated a novel lipid transporting protein from seeds of the garden fennel flower, *Nigella sativa.* The molecular mass, N-terminal amino acid sequence, and amino acid composition of the protein have been determined. The protein has a molecular mass of 9602 Da and contains eight cysteine residues which form four disulfide bridges.

3.3 Efficacy of Jellyfish against Two Tested Insects

Data in Table 6 demonstrated that mortality percentage increased with the increase of the doses of liquefied jellyfish for both tested insects. *O. surinamensis*, mortality percentage reached 96.67% when 3.5 % of digested jellyfish was applied. Otherwise, the mortality percentage was 100% when digested jellyfish applied against B. zonata larvae. The data presented that LC50 were 2.06 and 1.14 % for *O. surinamensis* and *B. zonata*, respectively.

Table 1. Effect of seed soaking in liquefied jellyfish solution on shoot, root and plant heights

Name of seed	Shoot		$LSD_{0.05}$	P	Root		$LSD_{0.05}$	P	Plant		$LSD_{0.05}$	P
	Control	Treated			Control	Treated			Control	Treated		
Anis	2.813^{a}±0.298	3.688^{a}±0.422	1.108	0.112 ns	1.438^{a}± 0.175	1.563^{a}±0.148	0.491	0.594 ns	4.250^{a}±0.433	5.250^{a}±0.473	1.375	0.141 ns
Canola	3.917^{b}±0.486	5.450^{a}±0.217	1.186	0.016 *	1.467^{a}± 0.264	1.017^{a}±0.241	0.797	0.237 ns	5.383^{a}± 0.616	6.467^{a}±0.438	1.684	0.189 ns
Coriander	8.960^{a}±0.399	9.020^{a}±0.446	1.257	0.921 ns	2.440^{b}± 0.198	4.590^{a}±0.465	1.062	0.0005 ***	11.35^{b}± 0.511	13.54^{a}±0.584	1.630	0.011 *
Cumin	2.313^{b}±0.092	3.188^{a}±0.092	0.277	0.000 ***	1.125^{a}± 0.125	1.500^{a}±0.134	0.392	0.060 ns	3.438^{b}± 0.148	4.688^{a}±0.188	0.512	0.0001 ***
Dill	8.500^{b}±1.088	12.18^{a}±1.056	3.185	0.026 *	2.450^{a}± 0.189	4.520^{a}±1.372	2.910	0.153 ns	11.85^{b}± 0.778	16.70^{a}±0.528	1.976	0.0001 ***

*Each value is the mean of ten replicates, **±:** Standard Error, a,b,c: Significant among treatment; Means in the same row followed by the same letter are not significant different at $p < 0.05$. $LSD_{0.05}$: Low standard diviasion.*

Table 2. Protein molecular weight of marker and liquefied jellyfish solution

Band No.	Marker			Jellyfish		
	Rf	Raw volume	MW kDa	Rf	Raw volume	MW kDa
1	0.018	473	260	0.011	660	282
2	0.058	446	140	0.185	64	52
3	0.097	320	100	0.24	58	44
4	0.166	399	70	0.385	31	38
5	0.245	802	50	0.476	34	38
6	0.372	160	40	0.938	111	38
7	0.437	479	35	-		-
8	0.549	102	25	-		-

M.W: Molecular weight

Table 3. Effect of seeds priming in liquefied jellyfish on protein bands of canola seedlings

Band No.	Canola					
	Control			Treated		
	Rf	Raw volume	MW kDa	Rf	Raw volume	MW kDa
1	-		-	0.007	591	298
n.p	0.011	519	282	-	51	-
2	0.173	91	55	0.158	30	60
3	0.206	77	48	-	76	-
n.p	-		-	0.377	591	38
4	-		-	0.901	51	38

M.W: Molecular weight; n.p: new protein band

Table 4. Effect of seeds priming in liquefied jellyfish on protein bands of coriander seedlings

Band No.	Coriander					
	Control			Treated		
	Rf	Raw volume	MW kDa	Rf	Raw volume	MW kDa
1	0.015	2013	267	0.015	1767	267
2	0.222	149	46	0.222	398	46
3	-	-	-	0.936	127	38

M.W: Molecular weight

Table 5. Effect of priming in liquefied jellyfish on protein bands of dill seedlings

Band No.	Dill					
	Control			Treated		
	Rf	Rawvolume	MW kDa	Rf	Raw volume	MW kDa
1	-		-	0.011	515	282
n.p	0.015	494	267	-	-	-
2	0.18	99	53	0.172	72	56

M.W: Molecular weight; n.p: new protein band created as a result of the treatment

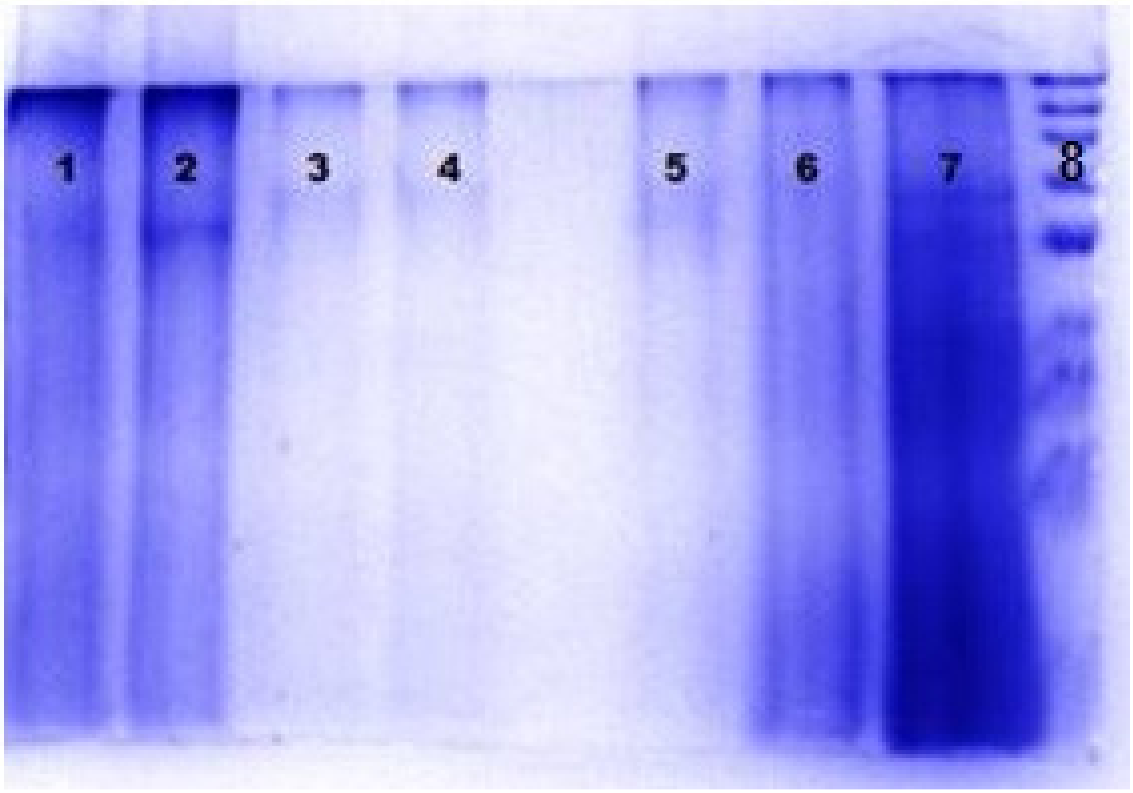

Fig. 1. Electrophoretic protein pattern of canola, coriander, dill, jellyfish and marker protein

Lan 1,2: Coriander; Lan 3, 4: Dill; Lan 5,6: Canola
Lan7:liquefied jellyfish; Lan 8:Marker protein
1, 3, 5 represent control samples that soaked in water
2, 4, 6 represent samples that soaked in liquefied jellyfish solution

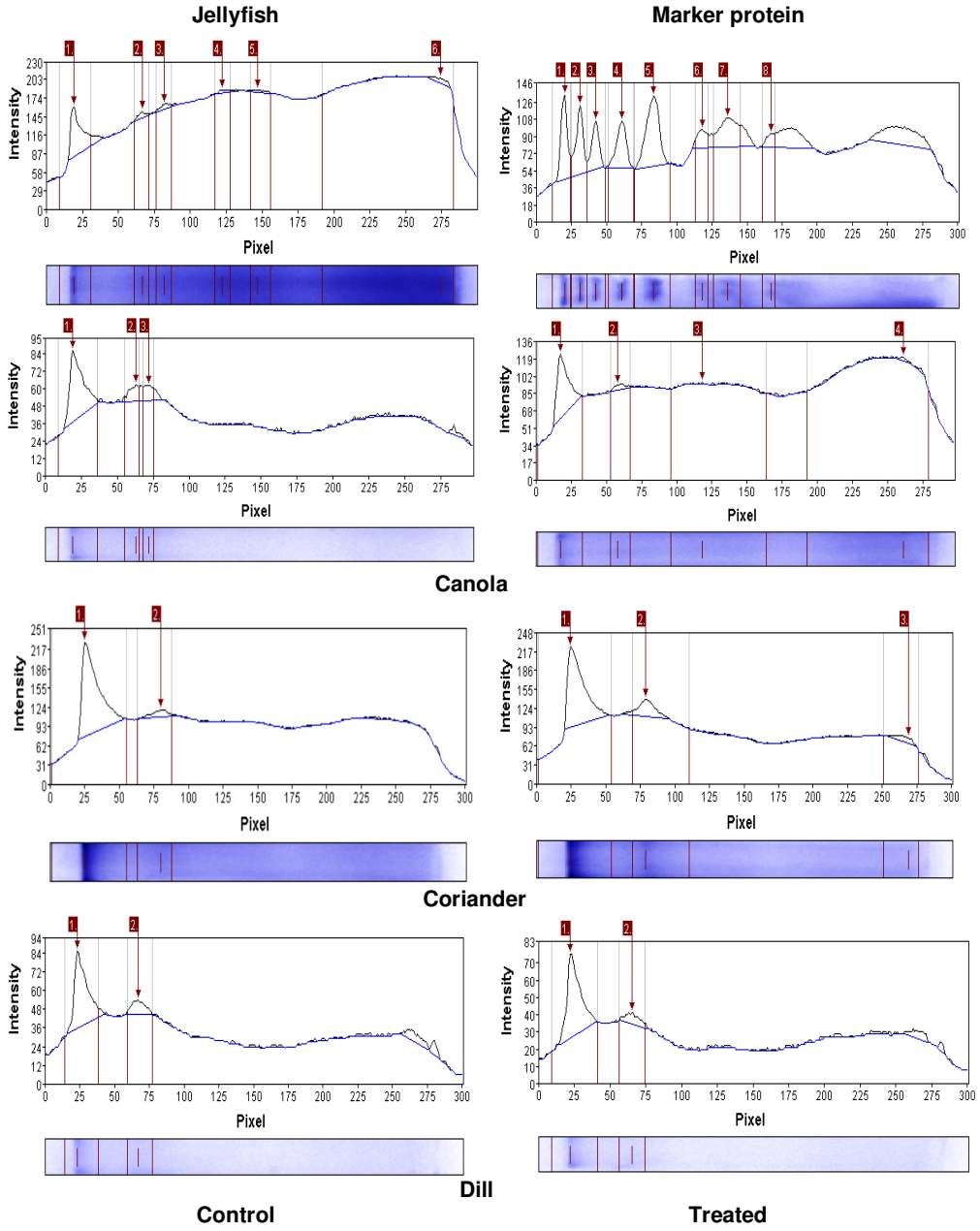

Fig. 2. Histogram of protein pattern of jellyfish, marker protein, canola, coriander and dill

Many researchers had documented the insecticidal effects of jellyfish full venom on three pest species, *Stephanitis pyri* Fabriciusa, *Aphis medicaginis* Koch, and *Myzus persicae* Sulzer. They recorded that the most potent toxicity against *S. pyri* Fabriciusa, and the corrected mortality recorded at 48 h was 97.86% [34]. Studies on the insecticidal properties of the jellyfish showed the presence of haemolytic activity and α-chymotrypsin-like serine protease [35], [36]. Also, the insecticidal activity of Jellyfish can be due to the presence of phospholipase A2 which had important role in the regulation of lipid metabolism [37] and led to decrease membrane phospholipids, membrane lipid peroxidation and finally decreased membrane integrity caused insect death [38].

Table 6. Susceptibility of *Oryzaephilus surinamensis* and *Bactrocera zonata* to digested jellyfish solution

Conc. (%)	Corrected Mortality %	
	O. surinamensis	***B. zonata***
Control	0	0
0.5	0	16.67
1	10	33.33
1.5	26.67	60
2	43.33	76.67
2.5	60	93.33
3	86.67	100
3.5	96.67	-
LC_{50}	2.06(Lower 1.78 – Upper 2.77)	1.14(Lower 0.95 – Upper 1.32)
Slope	5.24±0.695	3.52±0.47

4. CONCLUSION

The mentioned results expressed that Jellyfish can be used as growth promoter by seed priming in liquefied jellyfish and as bio-insecticide. But to support the results obtained it need to apply on large scales.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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