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Fruit seeds: A new bio-pesticide for *Spodoptera littoralis* (Boisd) control

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Abstract
Dried fruit seeds; i.e. date pits powder of date palm trees (*Phoenix dactylifera* L.) swia variety, wild custard apple seeds of *(Annona senegalensis* P.) mandarin seeds, of (*Citrus reticulate* B.)*;* considered as new bioactive compounds. They were used, as dust application, to control cotton leafworm, *S.littorais* (Boisd.).The biological studies and influence of this natural compounds on the growth and development of 2nd and 4th instar larvae were investigated. The lethal time of larval mortality recorded after 24h, 48h, 72h, 7days and 2 weeks after larval treatment with seed powders. All treatments caused higher mortality in the 2nd than in the 4th instar larvae, compared with zero% mortality in the control. Larval growth inhibited, and pupation drastically reduced to 72, 60, 58%, after treatment with date pits, custard apple and mandarin, respectively, compere with 98% in the control. Moreover, this new bio-pesticide had a latent effect, until the emergence of adult moths, with many new scores of malformations. Samples of the treated larvae were electrophoretically examined and the total body proteins of the 4th instar larval, treated with these compounds, performed by SDS-PAGE. Control and different treatments were separated into 24 different bands according to their relative frequencies (Rf values), and molecular weights (MW). The strong efficacy of the treated compounds on protein gel electrophoresis led to detection of new bands, and disappearance of some bands in comparison to control sample. Therefore, we recommend the use of this natural materials, as dust application, for the control of insect pests on their hosts under agricultural fields conditions.

Keywords: *S.littorais*, fruit seed, date, custard apple and mandarin seeds, bio-pesticide, protein gel electrophoresis.

Introduction
The cotton leafworm, *S. littorais*, causes great loss for many agricultural and vegetable crops in Egypt and other countries of the world. The use of different pesticides against this pest, for the last several decades, make it more resistant to traditional insecticides and more harmful to major strategic crops. Excessive use of pesticides decreased the population of natural enemies in the Egyptian agricultural systems. On the other hand, pesticides are very dangerous and toxic to humans, animals and other living organisms (Adel, 2012) [2]. The global amounted production of dates in 2010 season enhanced to 8.53 million metric tons, up from 7.53 million metric tons, while in 2015 season the most important crops worldwide are production of 156 million tons of citrus. (FAO, 2011& 2017) [16, 19]. Egypt is the highest country in date production, with an amounted of 1.56 million metric tons every year (Elbana, 2015) [11]. The most common varieties in Egypt, Mabrook, Zahidi, Aseel, Lasht, Dhakki, Majdool, Halawi, Bamy and Deggla (Khalid *et al.* 2016) [31]. During the period 2000 to 2014, the average amount of Egyptian exports of dates amounted 169 thousand tons, representing about 1.4% of the average amount of global exports, and about 2.4% of the average amount of Arab exports of dates. The average Egyptian exports of dates value amounted to about 8.99 million US dollars, accounting for about 1.71% of the average value of world exports, and about 2.5% of the average value of Arab exports of dates, Esmaeil (2017) [15].
Date pits powder consider as a good formulation activated carbon, which used as a novel bioadsorbents and effective adsorbent in various industries for polluted water with organophosphorus pesticide (profenofos) remediation from water (Yaish and Kumar 2015)[46].Date fruit contain many of antioxidant compounds and also an important source of vitamins, fibers ,dietary carbohydrates, lipids, protein,polyphenol,oleic,fatty acids, palmitic, lauric, myristic, stearic and linoleic acids.Al-Humaid *et al.* 2010 [4]; (Hossain, *et al.,* 2014, Khalid *et al*. 2016 & Ahmad and Imtiaz 2019 and Hassana, *et al.,* (2020) [27, 31, 3, 26]. The mandarins fruits (*Citrus reticulata*) are a rich source of bioactive compounds (secondary metabolites) such as flavonoids, terpenes, carotenes, linalool, namelycoumarins and mandarins peels are contain essential oils have a natural antimicrobial properties and insect repellents.(Mondello *et al*., 2005 & Viuda-Martos *et al*., 2008 and Garmendiaa, *et al.,* 2019 & El Sawia, *et al.,* 2019) [35, 43, 20, 14]. Crude seed extracts of Annona spp., showed more efficacy as insecticide to control *Plutella xylostella* L., compared with pyrethrum, that used as botanical insecticide. Leatemia, and Isman (2004) [33]. The pharmacological and phytochemical analyzed of two species *Annona senegalensis* and *A.cherimola* showed many of compounds like phenolic, alkaloids, tannins, flavonoids, glycosides, saponins, carbohydrates, proteins, phytosterols, and amino acids, steroid, carbohydrate, fat, crude fiber ,moisture, ash and other major mineral composition that, iron , calcium , lead , zinc, potassium, copper, magnesium, manganese and chromium. (Yisa *et al.,* 2010 & Jyothi *et al.,* 2011) [45, 28]. The laboratory studies used a mixture of leaves powder of many plants such as *Annona squamosa* (L.), *Eucalyptus globulus* (Labill.) and *Moringa oleifera* (Lam.) against the important stored grain pest *Tribolium castaneum* (Herbst.), the rate of mortality percentage reached 100 % with the increase in concentrations and also the development of

larval stage did not grow well and the moulting process were not complete to the next stage. (Anita *et al.*, 2012) [6]. Abortifacient properties of insecticidal from seed extracts from custard apple kernels,*A. squamosa*, was more effective against crop pests like fall army worm *S. frugiperda*, cotton leafworm *Spodoptera litura*(Fab.), cotton boll worm *Helicoverpa armigera* (Hubner) and red cotton bug *Dysdercus koenigii*(Fab.) Khalequzzaman, and Sultana 2006) [29] & (Khamis *et al.*, 2016) [30]. In Africa, wild custard, *A.senegalensis* (family:Annonac), also known as apple, had a lot of biological activities as an insecticide, antioxidant,antimicrobial,anti-inflammatory, antispasmodic, spermatogenic and analgesic activities. Okhale *et al.*, (2016) [38]. The biological activity study and bioassay of leaves of *Annona cherimola* Mill found to have cytotoxic, antitumoral, antiparasitic, pesticidal and inmunosupresive activities, the most active extracts were selected and evaluated against wheat coleoptile bioassay. Gutiérreza *et al.*, (2017) [25]. The toxicity of ethanol and hexane seed extract, of *Annona squamosa*, was tested against *S. litura*. the results showed that mortality increased at all doses up to 96 hrs. of exposure. Vetal and Pardeshi (2019) [42]. Also, the third innovation seed of citrus peel powder were recorded as insecticidal affected that induces higher mortality, decreased reproductive,inhibition larval development, adult emergence and weight loss of *Tribolium castaneum* (Herbst). (Nta *etal.*,2017) [37]. Each year Globally, approximately 1.3 billion tons of food is lost as waste, amount to 50% of production is enough to feed 3 billion people, the worldwide annual food wasted raged US$310 & US$680 billion, in developing and industrialized countries, fruit and vegetable waste residues reached roughly 50 – 40% (fruit, tubers, and legumes), 30 -20% (cereals and oleaginous fruits), (Gustavsson *et al*. 2011) [24]. (FAO 2016) [18]. The current study aims to maximize the benefit of reused of three important economic fruits (date pits, custard apple seeds and mandarin seeds) and evaluate the efficacy of seeds powder of this fruits as a new bio-pesticide for the 2nd and 4th larval stages of cotton leafworm, *S. littorais*.

Materials and Methods

1. Samples of seeds powder

Our experiment was in October, 2019, a new natural materials of fruit seeds waste: date pits, Annona and mandarin seeds were collected from different market in Egypt. after that samples of date pits seeds were washed with distilled water, then it was roasted under a temperature of 40°C for 24h next. Seeds of Annona and mandarin were separated then it washed with distilled water and it was dried at 40ºC for 2 days in an air oven, finally all fruit seeds of samples were crushed individually in a hand grinder. Hassana *et al*., (2020) [26].

2. Bioassay bioactive compounds against *S. littoralis.*

a. Insect culture:

The cotton leafworm *S. littorais* strain were were reared on fresh castor leaves, *Ricinus communis* L., it obtained from a sensitive laboratory culture were reared under constant temperature of 25±5Cº and R.H 65±5% at Cotton Leafworm Researh Department, Plant protection Research Institute.

b. Toxicity assays

Four treatments using the seed dust of the follow plants:

1. Date pits of Siwa variety *Phoenix dactylifera* L. (T1).
2. Wild custard apple seeds of *A. senegalensis* (T2).
3. Mandarins seeds of *C. reticulate* (T3).
4. Control" Distilled water" (T4).

The toxic and biological effects of three different fruit seeds (date Pits of Siwa variety *Phoenix dactylifera* L. (T1), wild custard apple apple seeds of *A. senegalensis* (T2) and mandarins seed of *C. reticulate* (T3), were evaluated. Starved larvae of *S. littoralis* were collected from colony. Fifteen larvae of 2nd and 4th instar were kept in glass jars (5 replicates of each jar/ treatment). Castor oil leafs were washed by water 1day before of the experimental, then airbrushed by water. A limited amount of dust seed (about 0.5 gm) from different seed powders of materials were dusted over the leaves, then dried for 3minuts in room temperatures. The treated and untreated leaves (control) were kept into jars, after that all jars were kept in the incubator at 25 ± 1°C. and 65±5% RH. The mortality percentage of larval was recorded after 24, 48,72 and 96 hours. Data was recorded after 7 and 14 days from the begin of the experimental. Observations were recorded until pupation and adult's emergency and separating the female's pupa from males.

3. Histological studies

Our experiment aimed at the study of the biochemical and histological characterization of 4th instar of *S. littoralis*, larvae after 7 days feeding on treated leaves.

4. Protein by Electrophoresis

Total protein were separat by using polyacrylamide gel electrophoresis(PAGE),the protein bands were visualized by staining with Coomassie Blue R250 stain, rinsed in distaining solution until the dark ground become colorless except for the blue protein bands According to the method Brad ford (1976)[8]. Davis (1964))[10] Laemmli, (1970) [32].

5. Data analysis

Analysis of variance (ANOVA) at 5 % level of significance was used (SAS Institute Inc., 1996). The denatured protein bands in polyacrylamide gel were analyzed using Quantity One software (Version 4.6.2) based on the method suggested by Nei and Li (1979) [36].

Results and Discussion

1. A Toxicity of the tested powder on 2nd instar larvae of. *S. littoralis*

Our results in table (Table 1) showed that, the effect of fruit seed powder on the growth and development of 2nd instar larvae of *S. littoralis*.

The highest percentage mortality was 28 % and 14 % after 24 hrs of treatment on mandarins (T3) and annona (T2) while date pits (T1) showed an effect after 48 h with 28% and also the total number of larval mortality of this stage after 14 days were 41, 42 and 82% for T3, T1 and T2, respectively. The effect of all treatment on pupal duration and pupal weight were difference at significant ($P<0.05$), compared with control, (table 1and Fig 1). The percentage of pupation decreased to 58, 60 and 72 % after larvae feeding on date pits of Siwa variety (T1), mandarins seed (T3) and wild custard apple seeds (T2) compare with control (98%). Therefore, highly significant effect of 2nd instar larvae were complete to pupal stage. The highest percentage of abnormal female were recoded after larvae feeding on T1 with 44% compare with zero in control.

Table 1: Bio- pesticide effect on the biological aspects of 2nd S.*littoralis* under laboratory conditions.

Biological aspect	Treatments					LSD
	T1	T2	T3	Control		
	Date–Pits	Annona	mandarins		F	
Pupal duration(day)	10.07 ± 0.01 a	10.58 ± 0.01 a	11.34 ± 0.10a	11.31 ± 0.14 a	10.36	0.41
Pupal Weight (mg)	304.29±0.39a	289±0.26a	318.86 ± 1.04 a	282.05 ±6.33 a	1.24	17.44
% Pupation	86	80	80	96		
% Adult Emergency	81	80	85	94		
% Pupal Mortality	19	20	15	2		
%♀ normal	91	20	90	41		
%♀ Abnormal	9	0	10	0		
% ♂ normal	46	60	57	58		
% ♂ Abnormal	54	4	43	0		
Sex ratio♀: ♂	1.2	1:3	1:2	1:1		

Means followed by different letters in each column are significantly different (P, 0.05)

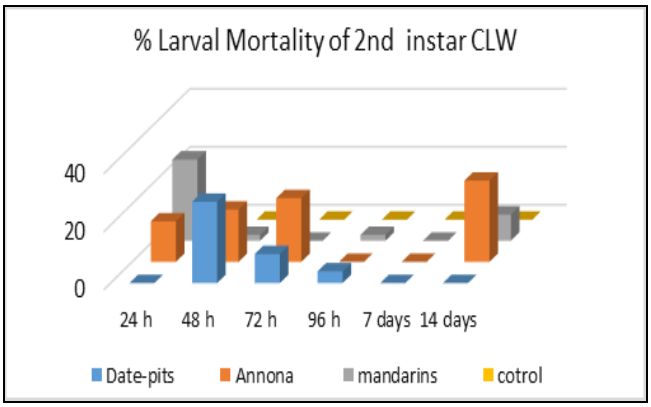

Fig 1: Percentage of mortality of 2nd instar larvae of S.*littoralis* after treatments.

This results agree with Chnimi *et al*., 2017[22], they recorded that, date palm fruits (*Phoenix dactylifera* L.) have richness in phenolic antioxidants, dietary fiber and also these fruits contain high amount of estrogenic compounds like isolated from date pollen, the estrogenic compounds may be effect on sex ratio and increased number of males moths like in our results as showed in table 1. Data illustrated in table 2 and fig (2) showed the highest mortality percentage was 20% when 4th instar larvae fed on T2 and T3, also no significant differences between all treatment of pupal duration and weight. Also, there were no adults (male and female) malformation recorded on control treatment, while the highest percentage of abnormal females were with T3 treatment (10 %), and abnormal male moths were 54 % for T1 and 43 % for T3, compare with 0% in the control.

Table 2: Bio- pesticide material effect on biological aspects of 4th instar larvae, S.*littoralis*, under laboratory conditions.

Biological aspect	Treatments					LSD
	T1	T2	T3	Control		
	Date–Pits	Annona	mandarins		F	
Pupal duration(days)	8.51 ± 0.01a	9.17 ± 0.3 b	9.18 ± 0.48b	10.55 ± 0.09 a	17.4	0.44
Pupal Weight (mg)	326.05±11a	297.0±11b	260.6±7.7 c	291.09 ±5.1 b	12.11	20.48
% Pupation	58	72	60	98		
% Adult Emergency	82	92	90	98		
% Pupal Mortality	20	8	10	2		
%♀ normal	39	36	33	41		
%♀ Abnormal	44	14	0	0		
% ♂ normal	65	49	57	57		
% ♂ Abnormal	46	16	11	0		
Sex ratio♀: ♂	1.1	1:1	1:1	1:1		

Significantly different (P, 0.05).

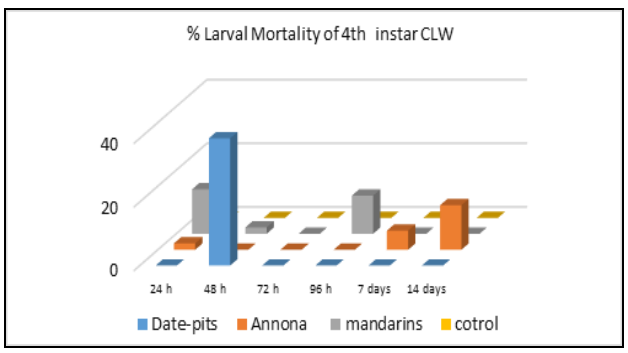

Fig 2: Percentage of mortality of 4th instar larvae of S.*littoralis* after treatments.

In Table (2) as mentioned above, the sex ratio of adult moths changed toward the increased number of male moths after treating the 4th instar larvae with T1, T2 and T3. This may be due to the latent effect in the old stage some hormone compounds on new compounds that effected on larvae development to adult moths, Protein gel results: 6 new bands appeared with T1 and some bands disappeared with T2, T3 as show in (Table 4 and Fig.5), this results agree with El-Mougy (1991) [13] who indicated that in rats, after treatment with date fruit extracts they induced an increase in sperm count due to effected on hormone. The effect of all treatment on pupal duration showed no significant difference. Pupation percentage were decreased to 58 and 60 % and 72 % when larvae fed T1, T3 and T2 compare with control 96%. The effect of all treatment on pupal duration showed no significant difference, the pupation percentage were affected and decreased to 86 and 80 % when larvae fed on T1, T2 and T3 compare with control 96% (Table2). From the result, the abnormal female and male reached up to 9, 10 % and 54 and 43% for T1 (date pits) and T3 (mandarins), this agree with Abdullahi and Muhammad 2004 [1], who reported that, the effect of

dried leaves and fruit powders on cowpea weevil (*Callosobuchus maculates*) induced mortality from 83 to100 %, the effect of seed extracts of *A. squamosa* inhibited 50% of larval growth for two insect pests *Trichoplusia ni* and *Spodoptera litura* (Leatemia and Isman 2004) [33]. The chemical properties study of two species of *A.senegalensis* and *Annona cherimola* recorded many phenolic compounds, tannins flavonoids, alkaloids and glycosides which plays important roles in body development and growth by Yisa *et al.,* 2010 [45] & Jyothi *et al.,* 2011 [28] Anita *et al*.,2012 [6] found that, when treated *Tribolium castaneum* with leaves of *A. squamosa*, *M. oleifera* and *E. globulus* separately larval mortality increased to 100% with increasing in concentrations and the larvae did not complete the growth development to the next stage. However, Nta *et al*., (2017) [37] demonstrated that treatments by sweet orange and tangerine seed powder induced higher significantly mortality and larval development caused of *T. castaneum* and also reduced adult emergence and weight loss. Some workers have observed that Hossain *et al.,* 2014 [27] who using date–pits powder as a bio-insecticide and chemical pesticides, also it contains activated carbon which purifying water by removing different types of pollutants such as dyes, heavy metals, boron and phenolic compounds, date pits powder is a rich source of phenolic, antioxidant potential ,fiber and polyphones which studied that on survival rate and all development stages of *Callosobuchus maculatus* .(Abdullahi, *et al.,* 2004 & Waly *et al.,* 2015 & Essa and Elsebaie 2018) [1, 44, 16]. The results agree with Vetal and Pardeshi 2019 [42] Whose recorded increased of 3rd instar larvae *S.litura* mortality after treatment with seed extract of *A. squamosal* increasing in concentration of plant extracts.

Table 3: New Scoring of larval–pupa – adult of S.*littoralis* after treated with fruit seed powder under laboratory conditions.

Scores	Characteristics	T1	T2	T3
		Date-pits	*Annona*	mandarins
0	Adults seemed to be normal	+	+	+++
1	Adults with wings slightly curled	+++	+++	+++
2	Adults wingless	+++	+	++
2*	Adults wing shortness and deformed			++++++
3	Adults severely curled	+	+++	
3*	Sharp curvature of the wings			+
4	Adults attached with puprium		++	+++
4*	Adhesion to the abdominal area		+	+++
5	Partial emergency (head and thorax)	+	+	+
5*	The antenna is deformed		+	+
6	Partial emergency with head only	+	+	+
6*[a]	Head sticking to the moulting seam		+	+
6*[b]	Abdominal adhesion	+		
7	Posteriorly partial emergency	+	+	
7*	End abdominal exit only			+
8	Dead pupa	+	+++	+
9	Larval pupal intermediate			
10	Dead larvae			

* New record by Dr. Samah Sayed Ibrahim

Table 4: Haemolymph protein pattern of 4th larval instar of S.*littoralis* : Relative fragmentation (Rf) and Molecular weight (Mw)

Parameters				T1	T2	T3
Band No.	Rf	Mw	Control	date-pits	mandarins seed powder	Annona seed powder
1	0.02	245	-	+	-	-
2	0.04	180	+	+	+	+
3	0.07	135	-	-	-	-
4	0.11	75	-	-	-	-
5	0.17	-	+	+	+	+
6	-	63	+	+	-	-
7	-	-	+	+	-	-
8	-	-	-	-	+	+
9	0.26	48	-	-	-	+
10	-	-	+	+	+	-
11	0.4	-	+	+	+	+
12	-	-	+	+	-	+
13	-	-	+	+	+	-
14	-	-	+	+	+	+
15	0.58	25	+	+	-	-
16	0.67	20	+	+	+	+
17	-	-	-	+	-	-
18	-	-	-	+	+	+
19	0.81	17	+	-	-	-
20	-	-	-	+	-	-
21	-	-	-	-	+	+
22	-	-	-	-	+	-
23	-	-	+	+	+	+
24	0.97	11	+	+	-	-

(+) = Present band
(-) = Absent band

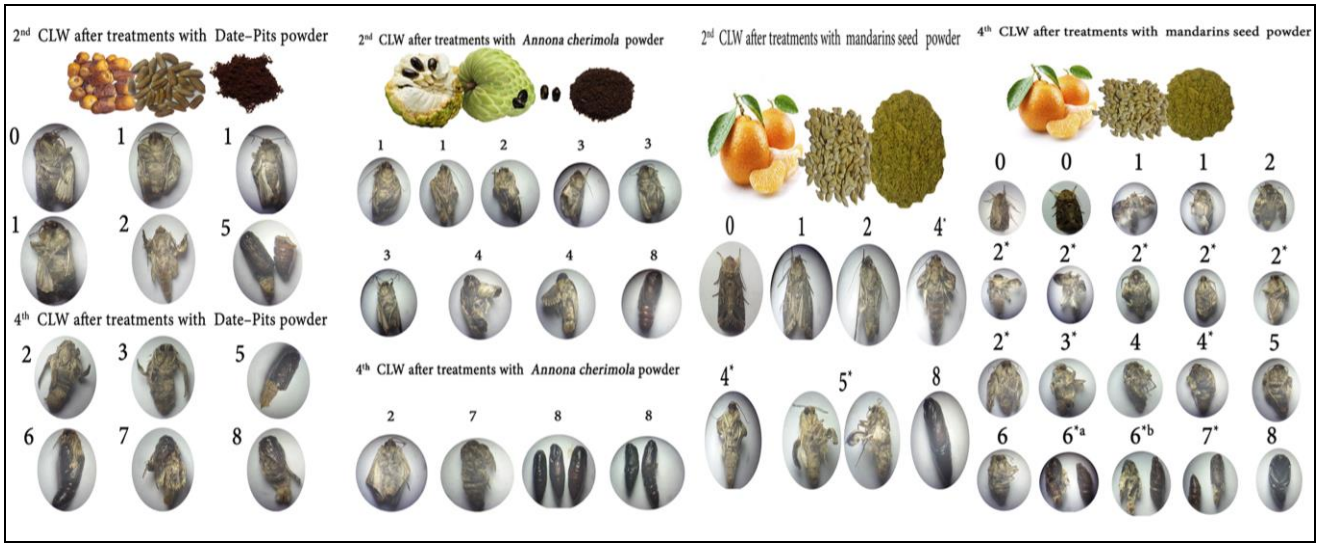

Fig 3: New bio-pesticide induce malformed of S.*littoralis*. Photos by: Dr. Samah S. Ibrahim

0: Adults seemed to be normal (control).
1: Adults with wings slightly curled
2: Adults wingless
2*: Adults wing shortness and deformed
3: Adults wing shortness and deformed
3*: Sharp curvature of the wings
4: Adults attached with puprium
4*: Adhesion to the abdominal area
5: Partial emergency (head and thorax)
5*: The antenna is deformed
6: Partial emergency with head only
6*[a]: Head sticking to the moulting seam
6*[b]: Abdominal adhesion
7: Posteriorly partial emergency
7*: End abdominal exit only
8: Dead pupa
9: Larval pupal intermediate
10: Dead larvae

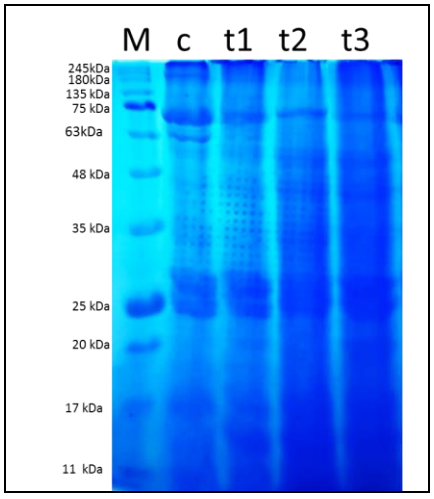

Fig 4: Electrophoretic protein pattern showing the differences in number and arrangement of bands among the treated groups with comparison to control. Separation of protein bands by gel electrophoresis.

In this section, we would like to shed some light on searching for a new strategy for pest control by dust application of fruit seed powder effects on the protein bands, this can be considered as a new strategy for an effective control approach of insect pests. Data on Table, 4 and Fig 6, showed the SDSPAGE protein electrophoretic pattern of the 4th instar larvae of S. *littoralis*, protein samples recorded a maximum number of 24 bands, which were not present in all of the treatment samples, at approximately Relative Mobility(Rf) ranging between 0.02 with molecular weight (Mw) 245 KDa and 0.97 with Mw 11 KDa. Our results indicated that, the new bio-pesticide caused more mortality and malformation to adult moths and also a disturbance in the sex ratio, this results were clear on table 4, Two bands (no 1 and 17) appear on T1 with a molecular weight of 264.63 and 18.74 KDa and intensity of 194.5 and 175.24, respectively. While two bands (no 6 and 7) disappeared on T2 and T3. Band no 19 only disappeared on all treatments compared with control. On the other hand, some bands created on T2 (no 8 and 22) while other bands (no11and 23) disappeared. Also, treatment with T3 resulted in five specific bands that were disappeared (6,7,12,15,19 and 24), compare with control, as a result of treatment. This may be considered as insect defense. In this section, we would like to shed some light on searching for a new strategy for pest control by dust application of fruit seed powder effects on the protein bands, this can be considered as a new strategy for an effective control approach of insect pests. These results were in accordance with Bakr *et al.* (2002) [9] whom tested the effect of four plant extracts on native protein and SDS-separated protein obtained from the larvae haemolymph of *S. littoralis* after treated.

The results coincided with that found by El-Bermawy (2004) [12] who analyzed esterases from body extracts of 6th larval instar and newly formed pupa of *S. littoralis* which treated with different botanical extracts treated, results show that any unusual increase or decrease in the activities of the enzymes in treated samples might be interpreted, on the molecular level, to depression or mutation of the regulating genes responsible for biosynthesis of polypeptide chains building these enzymes. The cytotoxic effects of phenolic and other antifeedant compounds on our innovation bio-pesticide mainly depend on their reactivity, phenols undergo radical reactions causing lipid peroxidation in the cell membrane and damage to the mitochondria, nucleus membranes, endoplasmic reticulum, and biochemical components such as nucleic acids and enzymes, these results are agreement with many authors (Randhir *et al.*, 2004;

Michałowicz and Duda, 2006) [40, 34]. (Al-Nagar *et al.*, 2019) [5] found a lot of compounds such as monoterpenes, sesquiterpenes and phenylpropenes that caused strong growth reduction to higher than 80% in the 2nd and > 70% in the 4th larval instar. The results also agree with Ghoneim *et al.*, 2021 [21] who observed the botanicals inhibit the immune capability, leading to the insects become susceptible to the action of pathogenic microorganisms and ultimately death.

Other experimental findings such as decreased pupation, pupal weight, and adult emergence could be explained by the absence of protein bands in treated insect samples, which could have induced by the toxic action of tested agricultural waste extracts, which inhibited the synthesis and protein expression. On the other hand, the appearance of new protein bands may be attributed to an increase in protein synthesis.

Table 5: Data of electrophoretic protein pattern showing physiological differences among the treated groups with comparison to control

Marker				C				T1				T2				T3			
Rf	**Mwt**	**Int.**	**B%**	**Rf**	**Mwt**	**Int.**	**B%**	**Rf**	**Mwt**	**Int.**	**B%**	**Rf**	**Mwt**	**Int.**	**B%**	**Rf**	**Mwt**	**Int.**	**B%**
0.02	245.00	37.88	3.78	-	-	-	-	0.02	264.63	194.15	6.28	-	-	-	-	-	-	-	-
0.04	180.00	44.46	4.43	0.03	204.67	126.30	6.53	0.04	175.91	162.1	5.25	0.04	177.94	115.41	4.91	0.05	164.17	186.76	7.23
0.07	135.00	41.33	4.12	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
0.11	75.00	116.31	11.60	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
-	-	-	-	0.14	69.38	132.31	6.84	0.13	70.16	162.95	5.27	0.12	72.27	145.24	6.18	0.13	70.68	186.02	7.20
0.17	63.00	36.64	3.65	0.18	61.18	82.60	4.27	0.18	61.44	116.19	3.76	-	-	-	-	-	-	-	-
-	-	-	-	0.21	56.04	54.09	2.80	0.21	56.27	111.26	3.60	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-	-	-	-	0.23	52.19	178.22	7.58	0.24	51.97	198.9	7.70
0.26	48.00	31.51	3.14	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
-	-	-	-	0.29	45.42	56.08	2.90	0.30	44.10	128.42	4.16	0.31	43.39	199.08	8.47	0.31	42.55	238.71	9.24
0.4	35.00	40.06	3.99	0.39	35.92	87.84	4.54	0.40	35.23	125.35	4.06	-	-	-	-	0.39	35.46	243.29	9.42
-	-	-	-	0.42	33.42	92.67	4.79	0.44	32.74	129.32	4.18	0.42	33.51	226.82	9.65	-	-	-	-
-	-	-	-	0.51	28.65	156.77	8.10	0.52	28.28	210.31	6.81	0.52	27.92	239.38	10.19	0.52	28.28	253.78	9.83
-	-	-	-	0.53	27.71	179.29	9.27	0.54	27.21	236.88	7.67	0.55	26.59	246.43	10.49	0.58	25.32	254.86	9.87
0.58	25.00	140.44	14.00	0.59	24.83	152.90	7.90	0.58	25.06	217.72	7.05	0.58	25	235.01	10.00	-	-	-	-
0.67	20.00	109.82	10.95	0.67	20.07	123.03	6.36	0.66	20.57	172.63	5.59	0.77	17.77	254.38	10.82	0.67	20.35	255	9.87
-	-	-	-	-	-	-	-	0.73	18.74	175.24	5.67	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	0.79	17.41	208.27	6.74	-	-	-	-	0.79	17.23	255	9.87
0.81	17.00	164.94	16.45	0.82	16.56	211.58	10.94	-	-	-	-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	0.83	16.06	230.84	7.47	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.85	14.89	255	9.87
-	-	-	-	-	-	-	-	-	-	-	-	0.87	14.45	255	10.85	-	-	-	-
-	-	-	-	0.91	12.7	227.85	11.78	0.91	12.90	253.71	8.21	-	-	-	-	0.92	12.37	255	9.87
0.97	11.00	239.48	23.88	0.98	10.71	251.17	12.98	0.98	10.63	255.00	8.25	0.97	10.84	255	10.85	-	-	-	-

Rf: Relative Mobility, Mwt: Molecular Weight, Int.: Intensity, B%: Band Percent

Table 6: Data of Similarity Index (SI%) and Genetic Distance (GD%) showing the differences in electrophoretic protein pattern among treated groups with comparison to control

		C	T1	T2	T3
		SI%			
C	GD%	-	70.90	22.30	36.70
T1		29.10	-	28.70	41.70
T2		77.70	71.30	-	41.30
T3		63.30	58.30	58.70	-

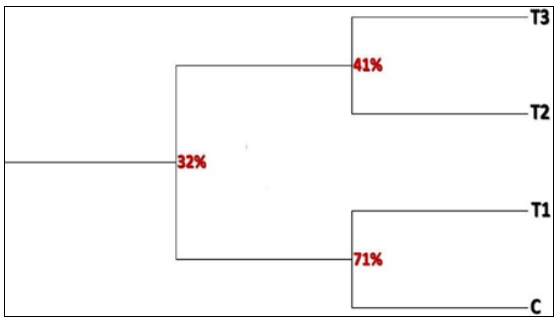

Fig 5: Dendogram showing relationship among the treated groups with comparison to control based on the electrophoretic protein pattern.

Depending on data listed in table 6 and Fig 5., The similarity index (SI) in DNA patterns indicates that, T2, T3 and T1 were recorded 22.30, 36.7 and 70.9 %, it means the treatment with Annona and mandarins induce high percentage of mutation in genome of *S. littoralis* that showed in Table 6.and illustrated with Dendogram in fig 5, the relationship between T1 and control was 71% while T2 and T3 was 41%. The main idea of this paper under investigation by Academy of scientific research& Technology, patent No. (888/2021).

Conclusion

This study showed that a small amount of dried fruit seed wastes,date pits powder of date palm trees (*Phoenix dactylifera* L.) swia variety, wild custard apple seeds of *Annona senegalensis* and mandarin seeds, *Citrus reticulata*, that we can used as dust application to control insect pests. In other words, any insect larvae feed on a small number of dried fruit seed wastes will be affected. The residual activity of dust formulations makes them ideal for field treatment by inducing mortality on the primer instar larvae and induced latent effect for the next generation by effecting the total protein in the next stages of target pests. These new bio-pesticides could be included in integrated pest management programs, against insect pest damaging major agricultural crops in Egypt, as an alternative to traditional chemical pesticides. This will save major crops from pest attack and prevent the pollution of the environment. However, further

studies are still needed in the future to fully understand the genes responsible for increasing the effectiveness of these bio-pesticide compound.

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