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Nano Formulation of Essential Oils Against *Agrotis ipsilon* (Hufn.) Larvae (Lepidoptera: Noctuidae)

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Abstract: The larvae of *Agrotis ipsilon* (Hufn.) is more commonly known as the black or greasy cutworm. It is a polyphagous serious insect pest of different economic plants found throughout the world. The present study aimed to evaluate the effect of two formulations, bulk and nano formulations the essential oils; purslane, mustard and castor against *Agrotis ipsilon* larvae. The toxicity effect of the essential oils purslane, mustard and castor were evaluated against the fourth instar larvae of *Agrotis ipsilon* while, the toxicity of their bulk and nano- formulations were tested against 2nd and 4th instar larvae under laboratory conditions at 26 ±2°C and 65 ± 5 % R.H. The results showed that purslane oil had the most toxic effect, compared with mustard and castor oil. The LC_{50} values of purslane or mustard were lower (0.42 and 0.70 ppm). The 2nd instar larvae were more sensitive to the nano-emulsion compounds compared to the 4th instar larvae.

Key words: Nano Formulations · Purslane · Mustard · Castor · Oils · *Agrotis Ipsilon* · Control

INTRODUCTION

Black cutworm (*Agrotis ipsilon* Hufnagel) is a pest that affects over 30 crops, including beans, broccoli, cabbage, carrot, spinach, eggplant, lettuce, potato, tomato and turnip, [1, 2]. *Agrotis ipsilon* larvae feed above ground and each one can consume over 400 cm^2 of foliage during its development. Larvae in the 4th instar may damage severely the stems of young plants and one larva may destroy the stems of several plants in a single night [3]. Insecticides derived from plants may be a safer alternative and could be used as a cost-effective means of pest control for farmers in developing countries if simple extracts can be prepared from readily available plants [4, 5]. Ghormade *et al.* [6] showed reduction in growth and development of *Helicoverpa armigera* and *Spodoptera litura* larvae when fed exclusively on castor leaves treated with Neem gum nano formulation (NGNF), Encapsulating nano-particle layers at the emulsion droplet interface may be engineered to increase droplet stability and control of release kinetics. Environmental mobility causes contamination of several environmental compartments [7-10]. Nanotechnology is used for the development of bio-pesticides, fertilizers and many other purposes [11, 12]. Nano particles have large surface areas, it can adsorb and bond with many compounds, circulate in insect/ lepidopteran systems[13]. Terpenes are insect antifeedant and toxic properties [14]. Alpha-pinene found in many essential oils activities such as repellent and antifeedant [15-19] uses of some insecticides of plant origin on *Scritothrips citri* Moulton (Thysanoptera, Thripidae) in redacting distortions orange fruits for export. Linalool is found in many flowers and spice plants, also exhibits fumigant and contact toxicant properties on pests of stored product, [20-23]. Formulations of nano are able to protect the botanicals from degradation [8]. Also, nano composites are effective in reducing leaching and in photo stabilization of the herbicides trifluralin [24, 25]. Emulsions formulation improved stability of water insoluble pesticide, b-cypermethrin [26]. Loaded microcapsules from garlic extract enhanced the toxicity to the stored product pests [27, 28]. Nanoparticles have a broad range of industrial as well as biological applications, [29]. Also, Silver nano paticles were used as an entomotoxic against *S. oryzae*, [11]. SNPs prevented the growth of *Bombix mori* nuclear polyhedrosis virus, [30, 31]. Silica nano particles were used for insect control as synergists of botanical compounds, [32]. Therefore,

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it is essential to reduce the use of synthetic pesticide sprays by alternate sprays of potent, environmental friendly and safe natural products. Essential oils are proved as potential sources of alternative compounds. The essential oils are known to be environment-friendly [33- 41].

The present study aimed to evaluate the effect of two formulations, bulk and nano emulsion of the essential oils; purslane, mustard and castor against *Agrotis ipsilon* larvae.

MATERIALS AND METHODS

Rearing Insect: Black cutworms, *A. ipsilon* were maintained for several generations in rearing units at 25±2°C and 65± 5%RH. in April 2020 at Plant Protection Research Institute. The adult moths were reared in glass jars measuring 10x25cm. A sucrose solution of 10% concentration was provided for feeding the moths. Females laid their egg masses on black muslins, newly-laid egg masses were collected. The old muslins were replaced by new ones and the adult moths were provided with fresh feeding solution. The newly laid eggs were categorized according to their oviposition date and were immediately placed in a suitable container. A small hair brush was used to transfer newly hatched larvae into plastic boxes measuring 10x25cm and containing a suitable amount of clean castor leaves. Larvae at the third instar were separated into individual plastic boxes to prevent cannibalism. Pupae were collected from the larval containers and were transferred to containers of sawdust.

Essential Oils (Bulk and Nano): Purslane, mustard and castor oils were used in the bioassay. They were obtained by steam distillation of dried plants [41]. The emulsions oils were prepared as follows: five drops of Triton X-100 as emulsifier were mixed thoroughly with 5 ml of each tested oil and then water was added to obtain the desired concentrations, (2%) in percent of (v/v). The emulsifier was mixed at the corresponding concentrations and used as check "Release mechanisms include dissolution, biodegradation, diffusion and osmotic pressure with specific pH" [42]. Encapsulated of three oils (Castor, Mustard and Purslane) and their nano emulsion were prepared by high-pressure homogenization of 2.5% surfactant and 100% glycerol [43]. Four concentrations were prepared (3, 1.5, 0.5 and 0.05 %) for each tested bulk essential oils and nano-essential oils, the tested concentrations were (1.0, 0.5, 0.05 and 0.005 %).

Tested Essential Oils: Purslane, Mustard and Castor oil using the method described [41].

Against *A. ipsilon* Larvae: Bioassays were carried out using Purslane, Mustard and Castor essential oils on 4th instar *A. ipsilon* larvae. Larvae were fed 100g of a semi-synthetic diet described by Shorey and Hale [44]. The diet was prepared using 500 g kidney beans, 30 g agar, 65 g yeast, 3 g sorbic acid, 5 g benzoic acid, 10 ml formalin and 10 g ascorbic acid. The kidney beans and agar were autoclaved in 600 ml of distilled water and were then ground with the other components, except ascorbic acid, which incorporated with the prepared media after it had cooled to the appropriate temperature. Each essential oil was included in a series of increasing concentrations. (0.005, 0.05, 0.5 and 1.0%). were incorporated as aqueous dilutions into 100 g of the semi-synthetic diet. This procedure was carried out immediately before gelling in order to avoid decomposition. Media treated with distilled water and a 100 µl of Tween 80 was used as control. The selected larvae were tested using 4 replicates per concentration per essential oil with 10 larvae in each replicate. Each replicate was housed in a glass tube 10 cm in length and was fed on 1 g of the treated diet. The larvae were incubated at 25 ± 2°C and 65± 5% RH. Larval mortality was recorded daily for 4 days after treatment and compared with the control larvae. The mortality percentage was corrected using Abbott's formula, Abbott [45].

Preparation of Nano-Formulations

Nano-Emulsions Preparation: To prepare, emulsions of purslane, mustard and castor essential oils, Tween 80 and distilled water were prepared using a modification of the method described by Jerobin *et al.* [46]. Purslane, mustard and castor essential oils were diluted with distilled water to a ratio of 2: 1 (oil to water). Two percent of Tween 80 was added as an emulsifier. The emulsion was then sonicated for 30 min. using an ultrasonic cleaner set (model WUC-DO3H 290W) set at 60 Hz. It was then resonicated for 1 min. using a high energy ultrasonication probe (model VCX750) set to 750W and 20 kHz and it was then resonicated again for 30 min. by the ultrasonic cleaner set under cooling conditions.

Characterization of Nano Emulsion

Electron Microscopy Examination: In order to study the morphological shapes and sizes of the prepared nano-formulations all nano-samples of oils were examined by Transmission Electron Microscope (TEM). The mean

particle sizes were 20-60 nm, 20- 90 and 20-90 nm for purslane, mustard and castor-oils, respectively. It was shown that purslane oil nano-particles were smaller in diameter than those of mustard oil. Similar results were obtained by Karthikeyan, *et al*. [49] as they prepared neem nano-emultion using Tween 20 as an emulsifier but of 1:3 ratios of neem oil and tween 20, respectively. They obtained nano-emulsion of size 31.03 nm. They found that larger emulsion droplets (251.43 nm) shifted to a smaller size of 31.03 nm with an increase of Tween 20 concentrations. At this ratio, the nano-emulsion was stable as the ensured [50, 51], reported that the addition of surfactant to nano-emulsion systems caused the interfacial film to condense and stabilize, resulting in small droplet size.

Diluted emulsion was done using distilled water by adding the surfactant to form the oil emulsion before drop wising in the alginate solution, Jerobin *et al.* [46].

Bioassay Tests: Bioassays tests were carried out on 2nd and 4th instar *A. ipsilon* larvae. Samples of 100 g semi-synthetic diet previously described [44] were treated. All prepared formulations were incorporated into the diet as aqueous dilutions at the desired concentrations during the preparation of the diet. Series of concentrations of each formulation were used to calculate the LC_{50} values. Such procedure was carried out just before gelling in order to avoid decomposition of the used materials. Media treated with distilled water mixed with 100 µl of Tween 80 was used as control. All concentrations were prepared according to the active ingredient content in each formulation. In case of 2nd instar larvae, 5 g of treated semi-synthetic diet for each concentration was added in plastic cups of 120 ml in capacity. Bioassay tests of purslane, mustard and castor essential oils were carried out using serial concentrations of oil containing 30, 60, 90 and 120 ppm for bulk oil. For nano-emulsion formulations, serial concentrations of 5, 10, 20, 30 and 40 ppm were added to the targeted medium. Ten of 2nd instar larvae were then transferred to glass tubes with four replicates, of 10 larvae /replicate. In case of 4th instars larvae, glass tubes of 10 cm height were used. One piece weighted 1 g of treated diet was cut by the cork borer and was added in each glass tube and each larva was transferred individually to each tube with four replicates, of 10 larvae /replicate. Bulk purslane, mustard and castor essential oils were prepared in the concentrations of 30, 60 and 120 ppm methanol. Nano- emulsion was prepared in concentrations of 30, 60, 90 and 120 ppm methanol. Those cups and glass tubes were incubated at 25 ± 2°C and 65 ± 5 % R.H. Larval mortality was recorded daily during 4 days after treatment and adjusted for control experiment and the mortality percentage was corrected using Abbott [45]. Concentrations mortality regression lines were plotted in form of log/probit relation and the LC_{50} values were calculated using Ld-p line program according to Finney [52].

Statistical Analysis: Data were analyzed using one-way analysis of variance (ANOVA) followed by Tukey test using SPSS software .

RESULTS AND DISCUSSION

Bioassay of Essential Oils Against, *A. ipsilon* Larvae: In order to determine the most effective essential oil to be converted to the nano form, the toxicity effect of three essential oils purslane, mustard and castor were tested against the 4th instars larvae of *A. ipsilon*. Their toxicity against the 4th instar larvae are given in Table (1). It was shown that purslane was the most effective essential oil and castor oil was the least effective oil. LC_{50} values were 0.42%, 0.70 % and 0.81 % for purslane, mustard and castor, respectively 4 days post treatment. The toxicity index values were 100, 59.44 and 50.11%, respectively. The relative potency values were 1.88, 1.15 and 1.1, respectively.

Purslane Oil on *A. ipsilon*: Data in table (2) showed the effects of adding different formulations of purslane oil either bulk, nano-emulsion at sublethal dose to artificial diet which fed to 2nd larval instar of *A. ipsilon*. Their effects on bionomics in comparison with control have been evaluated.

The larval duration was prolonged in the three treatments in comparing with that in control (18.72 days). Means of larval duration was 22.61 and 24.30 days for bulk and nano-emulsion, respectively. The percentages of larval mortalities were increased in the two tested formulations in comparison with control (5.50%), while the maximum percentage mortality (85.00%) was recorded in larvae treated with nano-emulsion, while bulk oil produced (11.00%). These results indicated that purslane oil at all formulations caused different categories of larval mortalities according to its formulation. The larvae fed with two formulations of purslane oil produced malformed larvae 11.00% and 18.18 % for bulk oil and nano-emulsion, respectively, while control produced 4.20%.

Table 1: LC_{50} and LC_{90} of 4th instar larvae of *A. ipsilon* 4 days post exposed to essential oils

Essential Oils	LC_{50} ml (Fudicial limits)	LC_{90} ml (Fudicial limits)	Slope	Regression	Toxicity index	Relative potency
Purslane	0.42 (0.35-0.50)	1.32 (0.86-1.80)	3.00±0.10	0.95	100	1.88
Mustard	0.70 (0.55-0.92)	1.80 (1.28-2.10)	3.00±0.50	0.93	59.44	1.15
Castor	0.81 (0.66-1.02)	2.30 (1.76-2.70)	2.95±0.10	0.91	50.11	1.1

Table 2: Effects of purslane oil nano-formulations on certain biological aspects of the 2nd instar larvae of *A. ipsilon*

	Formulations			
Parameter	Bulk oil	Nano-emulsion	Control	F
Larval duration (days)	22.61±0.41[b]	24.30±0.24[a]	18.62±0. 0[c]	39.70**
% Larval mortality (*)	11.00 18.33±0.45[c]	85.00 21.00±0.37[b]	5.50 12.76±0.67[d]	510.58**
% Malformations in larvae (*)	11.00 18.50±0.50[c]	18.18 24.72±0.32[b]	4.20 11.35±0.64[d]	260.16**
Pupal duration (Days)	11.99 ±0.21[a]	12.85±0.20[a]	10.00±0.26[b]	47.17**
Pupal wt./mg	382.42±7.73[a, b]	357.87±8.25[b]	409.00±10.33[a]	4.11*
% Pupal mortality (*)	19.99 26.00±0.87[c]	26.93 31.33±0.38[b]	7.50 15.35±0.94[d]	610.2**
% Malformations in pupa (*)	18 25.35±0.22[c]	28 31.90±0.45[b]	5 (12.33±0.87[d])	690.54**
Adult longevity (days)	6.55±0.52	6.00±0.21	7.22±0.11	4.12[NS]
Fecundity (egg/ Female	670.30±12.99[b]	580.13±22.55[b]	910.88±83.32[a]	10.10*
% Hatchability (*)	70.00 (56.40±1.43[b])	20.00 (24.87±0.53[c])	97.00 (82.00±1.04[a])	525.83**

(*) Arcsin transformation of percentage. NS=Non-significant. *Significant. **Highly significant. Each value represent the means of four replicates (each composed of 10 larvae) ±s.e. Values with different letters within the same row are significantly different (P. < 0.05) (ANOVA) (Tukey test).

Also, pupal duration was prolonged with regards to control treatment (10.00 days) while mean duration of the two treatments were 11.99 and 12.85 for bulk oil and nano-emulsion, respectively. A significant decrease was occurred in pupal weight in the two formulations in comparison with control (409.00 mg) while means pupal weight for the two treatments were 382.42 and 357.87 mg for bulk oil and nano-emulsion, respectively. Also, significant difference between mortality percentages of pupae was recorded in the two treatments.

The authors could be stated that adding purslane oil to 2nd instar larvae with different formulations caused prolongation in pupal duration, decrease in pupal weight, increasing in percentage of pupal mortality as well as increasing in percentage of pupae malformation. Rao [52], showed that the LC_{50} values for neonate and the second instar larvae of *Helicoverpa armigera* were 0.002% and 0.004 % when fed Neem Azal-treated cotton leaves continuously. The LC_{50} values were 0.005%, 0.02% and 0.03% for the first, second and third instar larvae of *H. armigera,* respectively when the exposure was limited to 48 h. [53], mentioned that Neemix reduced the adult longevities of *A. ipsilon* compared with control and this may be due to the reduction in their weights and inhibition of proteins, lipids and carbohydrates metabolism. Adult longevity in the treatments was lower than control (7.22 days), while means in the treatments were 6.55 and 6.00 days for bulk oil and nano-emulsion, respectively. Fecundity of adult females showed significant decrease in the two formulations of purslane oil in comparison with control treatment (910.88 eggs /female). The maximum decrease was recorded in nano-emulsion (580.13 eggs/female), followed by bulk oil (670.30 eggs /female). The percentages of hatchability were 20 % and 70% for nano-emulsion and bulk oil, respectively.

Effects of Mustard Oil on *A. ipsilon*: Data in Table (3) showed the effects of adding different formulations of mustard oil (bulk and nano-emulsion) at sublethal dose to artificial diet which fed to 2nd larval instar of *A. ipsilon*. The larval duration was prolonged in the two treatments in comparison with control (17.74 days). Means of larval duration were 30.22 and 32.75 days for bulk and nano-emulsion, respectively. The maximum percentage mortality (34.30%), was occurred in larvae treated with nano-emulsion, followed by bulk oil (19.20%). These results indicated that Mustard oil at all formulations caused different categories of larval mortalities according to its formulation. Treatment of Mustard oil formulations produced malformed larvae in the percentages of 6.10% and 12.33% for bulk oil, nano-emulsion, respectively.

Table 3: Effects of mustard oil nano-formulations on certain biological aspects of the 2nd instar larvae of *A. ipsilon*

	Formulations			
Parameter	Bulk oil	Nano-emulsion	Control	F
Larval duration (days)	30.22±0.31[b]	32.75±1.10[b]	17.74±0.20[c]	145.40**
% Larval mortality (*)	19.20 (25.99±0.90[c])	34.30 (35.07±0.38[b])	6 (12.99 ±0.98[d])	733.94**
% Malformations in larvae (*)	6.10 14.83±0.89[a, b]	12.33 19.87±0.95[a]	0 1.92±0.09[c]	8.89**
Pupal duration (Days)	14.00±0.75[a]	15.00±0.77[a]	12.00±0.21[b]	9.89**
Pupal wt./mg	269.97±9.55[b]	275.44±10.95[b]	412.00±12.22[a]	34.90**
% Pupal mortality (*)	57.33 (49.93±0.82[b])	58.77 49.95±0.85[b]	7.95 (16.49±0.84[c])	815.22**
% Malformations in pupa (*)	19.11±0.80[b, c]	32.30±0.66[a, b]	4.90±2.55[c]	21.90**
Adult longevity (days)	6.60±0.57[a, b]	5.20±0.52[b]	7.70±0.85[a]	5.52*
Fecundity(Egg/ Female)	599.45±129.84[a, b]	490.25±122.43[b]	1085.70±152.44[a]	5.94*
% Hatchability (*)	80 62.90±0.90[b]	37 36.99±0.95[c]	96 75.89±0.88[a]	1536**

(*) Arcsin transformation of percentage. NS=Non-significant. *Significant. **Highly significant. Each value represent the means of four replicates (each composed of 10 larvae) ±s.e.. Values with different letters within the same row are significantly different (P.< 0.05) (ANOVA) (Tukey test)

Also, pupal duration was prolonged with regards to control treatment (12 days) while mean duration of the two treatments was 14 and days for bulk oil and nano-emulsion, respectively. A significant decrease was occurred in pupal weight in the two formulations in comparison with control (412.00 mg) while means pupal weight for the two treatments were 269.97 and 275.44 mg for bulk oil and nano-emulsion, respectively. Also, significant percentage mortality between resulting pupae was occurred in two treatments. The highest mean percentage mortality was 58.77% for nano-emulsion followed by bulk oil 57.33%, while control treatment was 7.95%. The highest percentage was (32.30%) produced from nano-emulsion, followed by nano-emulsion treatment and bulk oil treatment (19.11%), while control treatment produced 4.90% malformed pupae.

Means longevity of treatments were 6.60 and 5.20 days for bulk oil and nano-emulsion, respectively. Fecundity of resulted adult females showed significant decrease in the two formulations of Mustard oil in comparison with control treatment (1085.70 eggs/female). The maximum decrease was occurred in nano-emulsion (490.25 eggs/female), followed by bulk oil (599.45 eggs/female). In table (3) showed significant difference between mean percentages of hatchability, being 37% and 80% for nano-emulsion and bulk mint oil, respectively.

The findings of the present work clearly indicated that the tested essential oils showed insecticidal properties and negatively affected on the oviposition and reproductive parameters of *A. ipsilon* adults, as well as on pupal stage.

Effects of Castor Oil on *A. ipsilon*: Data in Table (4) showed the effects of adding different formulations of castor oil (bulk and nano-emulsion) at sublethal dose to artificial diet which fed to 2nd larval instar of *A. ipsilon*. The larval duration was prolonged in the two treatments in comparison with control (17.74 days). Means of larval duration were 32.42 and 34.95 days for bulk and nano-emulsion, respectively. The percentages of larval mortalities were increased in the two tested formulations in comparison with control (6%). The maximum percentage mortality (32.30%), was occurred in larvae treated with nano-emulsion, followed by bulk oil (17.20 %).

Also, pupal duration was prolonged with regards to control treatment (12 days) while mean duration of the two treatments was 13.14 and 15.10 days for bulk oil and nano-emulsion, respectively. A significant decrease was occurred in pupal weight in the two formulations in comparison with control (412.00 mg) while means pupal weight for the two treatments were 275.44 and 277.66 mg for bulk oil and nano-emulsion, respectively. The highest mean percentage mortality was 55.43% for nano-emulsion followed by 56.44% for bulk oil, while control treatment was 7.94%. The percentage of pupae malformation showed significant difference between means. The highest percentage was (30.10%) produced from nano-emulsion and bulk oil treatment (19.00 %), while control treatment produced 4.90%.

Means longevity of treatments were 6.90 and 5.95 days for bulk oil and nano-emulsion, respectively. Fecundity of adult females showed significant decrease in the two formulations of castor oil in comparison with

Table 4: Effects of castor oil nano-formulations on certain biological aspects of the 2nd instar larvae of *A. ipsilon*

	Formulations			
Parameter	Bulk oil	Nano-emulsion	Control	F
Larval duration (days)	32.42±0.51[b]	34.95±1.30[b]	17.74±0.20[c]	145.60**
% Larval mortality (*)	17.20 (23.89±0.80[c])	32.30 (33.77±0.48[b])	6 (12.99 ±0.98[d])	730.74**
% Malformations in larvae (*)	6.00 14.00±0.19[a,b]	12.00 19.17±0.25[a]	0 1.92±0.09[c]	8.29**
Pupal duration (Days)	13.00±0.65[a]	14.00±0.67[a]	12.00±0.44[b]	9.80**
Pupal wt./mg	275.44±9.45[b]	277.66±10.85[b]	412.00±12.22[a]	34.80**
% Pupal mortality (*)	55.43 (49.23±0.62[b])	56.44 49.45±0.43	7.94 (16.29±0.64[c])	815.12**
% Malformations in pupa (*)	19.00±0.70[b,c]	30.10±0.60[a, b]	4.90±2.33[c]	21.50**
Adult longevity (days)	6.90±0.87[a,b]	5.95±0.55[b]	7.70±0.55[a]	5.50*
Fecundity (Egg/ Female)	602.55±131.64[a,b]	495.35±123.93[b]	1080.50±150.94[a]	5.94*
% Hatchability (*)	84 66.95±0.95[b]	40 37.99±0.99[c]	96 76.80±0.78[a]	1535**

(*) Arcsin transformation of percentage. NS=Non-significant. *Significant. **Highly significant. Each value represent the means of four replicates (each composed of 10 larvae) ±s.e.. Values with different letters within the same row are significantly different (P. < 0.05) (ANOVA) (Tukey test)

control treatment (1080.50 eggs /female). The maximum decrease was occurred in nano-emulsion (495.35 eggs/female), followed by bulk oil (602.55 eggs/female). The results obtained in table (4) showed significant difference between mean percentages of hatchability, being 40 % and 84 % for nano-emulsion and bulk oil, respectively.

CONCLUSION

The obtained results show that nano formulation of the essential oils have significant effects on the toxicity of the 2nd and 4th larval instars of *A. ipsilon*. Purslane-formulations were more potent in its larvicidal effect against *A. ipsilon* than mustard and castor nano-formulations.

REFERENCES

1. Boughton, A.J., L.C. Lewis and B.C. Bonning, 2001. "Potential of *Agrotis ipsilon* Nucleopolyhedrovirus for Suppression of the Black Cutworm (Lepidoptera: Noctuidae) and Effect of an Optical Brightener on-Virus Efficacy". J. Econ. Entomol., 94(5): 1045-1052.
2. El-Salamouny, S., M. Lange, M. Jutzi, J. Huber and J.A. Jehle, 2003. "Comparative study on susceptibility of cutworms (Lepidoptera :Noctuidae) to *Agrotis segetum* nucleopolyhedrovirus and *Agrotis ipsilon* nucleopolyhedrovirus". J. Invert Pathol., 84(2): 75- 82.
3. Muhammad, S., U. Farman, N. Muhammad, A. Muhammad, A.U.R. Saljoqi and Z. Muhammad , 2007. "Effect of various insecticides for the controlof Potato cutworm (*Agrotis ipsilon* Huf., Noctuidae:lepidoptera) atkalam swat." Sahad J. Agric., 23(2): 423-426.
4. Isman, M.B. and C.M. Machial, 2006. "Pesticides based on plant essential oils: from traditional practice to commercialization. In: Advances in phytomedicine: naturally occurring bioactive compounds." M. Rai and M.C. Carpinella, Eds. Elsevier, New York.
5. Amoabeng, B.W., G.M. Gurr, C.W. Gitau, H.I. Nicol, L. Munyakazi and P.C. Stevenson, 2013. "Tri-Trophic Insecticidal Effects of African Plants against Cabbage Pests" Plos One, 8 (10): 1371-1382.
6. Ghormade, V., V.D. Mukund and M.P. Kishore, 2011. "Perspectives for nano-biotechnology enabled protection and nutrition of plants". Biotech. Adv., 29: 792-803.
7. Mansour, S.A., 2008. Environmental impact of pesticides in Egypt. Rev. Environ. Contam. Toxicol., 196: 1-51. https://www.ncbi.nlm.nih.gov/pubmed/19025091.
8. Rechcigl, J.E. and N.A. Rechcigl, 2000. Biocontrol and biotechnologicalcontrol of insects pests. CRC Press LCC, pp: 101-117.
9. Chowdhurry, A., M.A. Alam, M.S. Rahman, M.A. Hossain and M.M. Rashid, 2009. Antimicrobial, antioxidant and cytotoxic activities of Citrushystrix DC. fruits J. Pharm. Sci., 8(2): 177-180.

10. Tawtsin, A., S.D. Wratten, R. Scott, U. Thavara and Y. Techadamrongsin, 2001. Repellency of volatile oils from plants against three mosquitovectors. Engl. J. Med., 26: 76-82.
11. Debnath, N., S. Das, D. Seth, R. Chandra, S.C. Bhattacharya and A. Goswami, 2011. Entomotoxic effect of silica nanoparticles against *Sitophilus oryzae* (L.). J. Pest Sci., 84: 99-105.
12. Scarfato, P., E. Avallone, P. Iannelli, V. De Feo and D. Acierno, 2007. Synthesis and characterization of polyurea microcapsules containing essential oils with antigerminative activity. J. Appl. Poly. Sci., 105: 3568-357.
13. Barik, T., B. Sahu and V. Swain, 2008. Nanosilica-from medicine to pest control. Parasitol. Res., 103: 253-258.
14. Gonzalez Comoma, A., A. Guadano, C.E. Tonn and M.E. Sosa, 2005. Antifeedant/insecticidal terpenes from asteraceae and labiatae species native to Argentinean semi-arid lands. Z Naturforsch, 60: 855-861.
15. Fan, L.S., R. Muhamad, D. Omar and M. Rahmani, 2011. Insecticidal properties of Piper nigrum fruit extracts and essential oils against *Spodoptera litura*. Int. J. Agric. Biol., 13: 517-522.
16. Wang, B., W.Y. Feng, M. Wang, T.C. Wang, Y.Q. Gu and M.T. Zhu, 2008. Acute toxicological impact of nano- and submicro-scaledzinc oxide powder on healthy adult mice. J. Nanopart. Res., 10: 263-276.
17. Owolabi, M.S., M.O. Oladimeji, L. Lajide, G. Singh, P. Marimuthu and V.A. Isidorov, 2009. Bioactivity of three plant derived essentialoils against the maize weevils *Sitophilus zeamais* (Motschulsky) and cowpea weevils *Callosobruchus maculatus* (Fabricius). EJEAF Che, 8(9): 828-835
18. Regnault-Roger, A.C., M. Hamraoui, E. Holeman and R. Theron, 1993. Insecticidal effect of essential oils from mediterranean plantsupon *Acanthoscelides Obtectus* Say (Coleoptera, Bruchidae), apest of kidney bean (*Phaseolus vulgaris* L.). J. Chem. Eco., 19(6): 1233-1244.
19. Salem, S.A., A.M.E. Abd-El Salam, Ahmed S. Reda, M.A. Abdel-Raheem and El F.M. Hawary, 2017. Evaluate and assess the use of some insecticides of plant origin against *Scritothrips citri* Moulton (Thysanoptera, Thripidae) in reducting distortions orange fruits for export. Bioscience Research, 14(2): 354-361.
20. Loh, F.S., R.M. Awang, D. Omar and M. Rahmani, 2011. Insecticidal properties of Citrus hystrix DC leaves essential oil against *Spodoptera litura* fabricius. J. Med. Plants Res., 5(16): 3739-3744.
21. Abdelgaleil, S.A.M., M.I.E. Mohamed, M.E.I. Badawy and S.A.A. El-arami, 2009. Fumigant and contact toxicities of monoterpenes to *Sitophilus oryzae* (L.) and Tribolium castaneum (Herbst) and their inhibitory effects on acetylcholinesterase activity. J. Chem. Ecol., 35: 518-525.
22. Kostic, M., Z. Popovic, D. Brkic, S. Milanovic, I. Sivcev and S. Stankovic, 2008. Larvicidal and antifeedant activity of some plant-derived compounds to *Lymantria dispar* L. (Lepidoptera: Lymantriidae). Bioresour Technol., 99(16): 7897-7901.
23. Dayan, F.E., C.L. Cantrell and S.O. Duke, 2009. Natural products in crop protection. Bioorganic & Medicinal Chemistry, 17: 4022-4034.
24. El-Nahhal, Y., T. Undabeytia, T. Polubesova, Y.G. Mishael, S. Nir and B. Rubin, 2001. Organo-clay formulations of pesticides: reduced leaching and photodegradation. Appl. Clay Sci., 18: 309-326.
25. Boehm, A.L., I. Martinon, R. Zerrouk, E. Rump and H. Fessi, 2003. Nanoprecipitation technique for the encapsulation of agrochemical active ingredients. J. Microencapsul, 20: 433-441.
26. Wang, L., X. Li, G. Zhang, J. Dong and J. Eastoe, 2007. Oil-in-water nanoemulsions for pesticide formulations. J. Colloid Interface Sci., 314: 230-235.
27. Yang, F.L., S.G. Li, F. Zhu and C.L. Lei, 2009. Structural characterization of nanoparticles loaded with garlic essential oil and their insecticidal activity against *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae). J. Agric. Food Chem., 57: 10156-10162.
28. Iconaru, S.L., A.M. Prodan, M. Motelica-Heino, S. Sizaret and D. Predoi, 2012. Synthesis and char acterization of polysaccharide-maghemitecomposite nanoparticles and their antibacterial properties. Nanoscale Res. Lett., 7: 576-783.
29. Majumder, D.D., C. Ulrichs, I. Mewis, B. Weishaupt, D. Majumder, A. Ghosh, A.R. Thakur, R.L. Brahmachary, R. Banerjee, A. Rahman, N. Debnath, D. Seth, S. Das, I. Roy, P. Sagar, C. Schulz, N.Q. Linh and A. Goswami, 2007. Proceedings of the international conference on computing: Theory and applications, Kolkata, India, March 5-7, IEEE Press, India, pp: 563.

30. Debnath, N., S. Mitra, S. Das and A. Goswami, 2012. Synthesis of surface functionalized silica nanoparticles and their use as entomotoxic nanocides. Powder Technol., 221: 252-256.
31. Rahman, M.F., M.K. Siddiqui and K. Jamil, 1999. Sub-chronic effect of neem based pesticide (vepacide) on acetylcholinestrase and ATPase in rat. J. Environ. Sci. Health, 34(5): 873-884.
32. Pathipati Usha Rani, Joish Madhusudhanamurthy and Bojja Sreedhar, 2014. Dynamic adsorption of a-pinene and linalool on silica nanoparticles for enhanced antifeedant activity against agricultural pests, J. Pest Sci., 87: 191-200.
33. Isman, M.B., 2006. Botanical insecticides, deterrents and repellents in modern agriculture and an increasingly regulated world. Annual Review of Entomology, 51: 45-66.
34. Malik, M.A., M. Manzoor, H. Ali, A. Muhammad, S. Ul Islam, M. Qasim, N. Ahmad, A. Idrees, A. Muhammad and H.S.A. Saqib, 2016. Evaluation of imidacloprid and entomopathogenic fungi, *Beauveria bassiana* against the red palm weevil *Rhynchophorus ferrugineus* (Coleoptera: Curculionidae), J. Entomol. Zool. Stud. JEZS., 262: 262-268.
35. Abdel-Raheem, M.A., Naglaa F. Reyad, I.E. Abdel-Rahman and A. Al-Shuraym Laila, 2016. Evaluation of some isolates of entomopathogenic fungi on some insect pests infesting potato Crop in Egypt. International Journal of Chem. Tech. Research, 9(8): 479-485.
36. Ismail, I.A., R.S. Abdel-Rahman and M.A. Abdel-Raheem, 2016a. Utilization of certain plant extracts and entomopathogenic fungi for controlling the black fig fly, *Lonchaea aristella* on fig trees. International Journal of Chem. Tech. Research, 9(4): 35-42.
37. Ismail, I.A., R.S. Abdel-Rahman and M.A. Abdel-Raheem, 2016. Economical evaluation of different treatments for Fig trees against longihorne beetle, *Hesperophanes griseus* (Coleoptera: Cerambycidae) on fig trees. International Journal of ChemTech Research, 9(4): 122-125.
38. Abdel-Rahman, I.E. and M.A. Abdel-Raheem, 2018. Using entomopathogenic fungi as bio agents control on the red palm weevil, *Rhynchophorus ferrugineus* (Olivier) (Coleoptera: Curculionidae), Journal of Entomology and Zoology Studies, 6(6): 387-390.
39. Mohamed Abdel-Raheem, Huda A. Alghamdi and Naglaa F. Reyad, 2020. Nano Essential oils against the red palm weevil, *Rhynchophorus ferrugineus* Olivier (Coleoptera: Curculionidae), Entomological Research, 50(5): 215-220.
40. Reyad, N.F., H.A. Al-Ghamdi, M.A. Abdel-Raheem and M.A. Al-Shaeri, 2020. The effects of botanical oils on the red palm weevil, *Rhynchophorus ferrugineus* olivier (Coleoptera: Curculionidae), Applied Ecology and Environmental Research, 18(2): 2909-2919
41. Guenther, E., 1961. "The essential oils."(4) Von Nostanand con. N. York, pp: 132-147.
42. Vidhyalakshmi, R., R. Bhakyaraj and R.S. Subhasree, 2009. Encapsulation the future of probiotics-A Review. Advances in Biological Research, 3: 96-103.
43. Sakulk, N.U., P. Uawongyart, N. Soottitantawat and U. Ruktanonchai, 2009. Characterization and mosquito repellent activity of citronella oil nano emulsion. International Journal of Pharmaceutics, 372: 105-111
44. Shorey, H.H. and R.L. Hale, 1965. "Mass rearing of the larvae of nine Noctuide species on a simple artificial medium". J. Econ. Entomol., 58: 522-524.
45. Abbott, W.S., 1925. "A method of computing the effectiveness of an insecticide". J. Econ. Entomol., 18: 265-267.
46. Jerobin, J., R.S. Sureshkumar, C.H. Anjali, A. Mukherjee and N. Chandrasekaran, 2012. "Biodegradable polymer based encapsulation of neem oil nano-emulsion for controlled release of Aza-A". Carbohydrate Polymers, 90: 1750-1756.
47. Lertsutthiwong, P., K. Noomun, N. Jongaroonngamsang, P. Rojsitthisak U. and Nimmannit, 2008. "Preparationof alginate nanocapsules containing turmeric oil". Carbohydrate Polymers, 74: 209-214.
48. Bouchemal, K., S. Briançon, E. Perrier and H. Fessi, 2004. "Nano-emulsion formulation using spontaneous emulsification: solvent, oil and surfactant optimization". International Journal of Pharmaceutics, 280: 241-251.
49. Karthikeyan, S., P.A. Jeeva, J. Jerobin, A. Mukherjee and N. Chandrasekaran, 2012. Formulation and characterization of nanoemulsion coatings from Azadirachta indica. Int. J. Chem. Tech. Res., 4(4): 1566-1570.
50. Kale, N.J. and L.V. Allen, 1989. Studies on microemulsions using Brij 96 as surfactant and glycerin, ethylene glycol and propylene glycol as cosurfactants. Int. J. Pharm., 57: 87-93.
51. Ghotbi, R.S., M. Khatibzadeh and S. Kordbacheh, 2014. Preparation of neem seed oil nanoemulsion. Proceedings of the 5th International Conference on Nanotechnology: Fundamentals and Applications Prague, Czech Republic, August 11-13; Paper: 150.

52. Rao, B.R., P. Rajasekhar, M. Venkataiah and N.V. Rao, 1995. Bio-efficacy of 'Neem Azal' (azadirachtin 10, 000 ppm) against cotton bollworm, Helicoverpa armigera (Hübner). Ibid, 19(4): 329-333.
53. Abd El-Rady, A.M. and M. Osman, Soad, 2005. Toxicological and biological effects of neem and Jojoba oils on the black cutworm, *Agrotis ipsilon* (Hufn) in the laboratory. Egypt J. Agric. Res., 83(3): 937-948.
54. Finney, D.J., 1971. "Probit Analysis. Cambridge University", Press, London, pp: 333.