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Nano bio pesticide for controlling cotton leafworm *Spodoptera littoralis* (Lepidoptera: Noctuidae)

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Abstract:

Nanoparticle compounds such as *Moringa oleifera* Lam. leaf powder, boiled white and brown eggshells powder for chickens and kapritia spring water form Siwa Oasis in Egypt, these natural materials were evaluated for first time under laboratory conditions against the 4th instar larvae of cotton leafworm *Spodoptera littoralis* (Boisd.) (Lepidoptera: Noctuidae). The results investigated that new nanoparticles of all eggshells and *M. oleifera* powder and kapritia water affected on biological aspects of target pest such as, larval mortality were recorded that,١٠, ٨, ٦ and 4% for *M. oleifera*, kapritia water, brown and white eggshells, respectively, compared with control 1%, the intermediated shapes were recorded during this studied. Also adults moths emergency percentage of *S.littoralis* are decreased to 89 , 82, 73 and 65 % after treatment with white eggshells, brown eggshells , *M. oleifera* leaf powders and kapritia Siwa water, respectively, compared with control 93%. Subsequently, adults malformed increased after all treatments; fecundity (Eggs/ female), fertility and also were affected. These compounds considered as nanoparticle, these innovation technologies to enhance our country developmental strategies in order to achieved sustainable agriculture and integrated crop management by alternative insecticide, environmental protection form pollutant, increase natural enemies for insect and food safety for better human healthy for a new generation.

Introduction

The Egyptian cotton leafworm *Spodoptera littoralis* (Boisd.) (Lepidoptera: Noctuidae) is the most dangerous pests in Egypt and Africa, causing significant economic loss of cotton, tomato, lettuce, strawberry and other vegetables crop in both greenhouses and open fields (Abd El-Razik and Mostafa, 2013 and EPPO, 2014). *Moringa oleifera* Lam. is medicinal plant with many pharmacological properties, leaves are a nutrient-dense food with high concentrations of protein, carbohydrates, fiber and a good source of several vitamins and minerals such as vitamins A, B, C, D, and E; folic acid; nicotinic acid; iron; calcium; zinc; potassium; magnesium and copper (Singh and Singh, 2019 and Walia *et al*., (2019). The chemical constituents of *M.oleifera* stems, leaves, flowers, pods and seeds are alkaloids, phenolicacids, gallic,

chlorogenicacid, ferulicacid, glucosinolates, flavonoids, quercetin, vanillin and kaempferol, tannins, steroids, coumarins, saponins, quinones and resins such as which have nutritional, pharmaceutical and antimicrobial properties (Anwar *et al*., 2007; Mbikay , 2012 and Mensah *et al*., 2012). Siwa Oasis (29.12° N, 25.43° E) is an isolated location in the Western Desert of Egypt, approximately 330 km from Matrouh City situated in the Northern Mediterranean Coastal Zone, chemical and radioisotopic constituents of ground water resources were Cl-, SO42-, CO32-, HCO3-, Na+, K+, Ca2+ and Mg2+, Al, B, Ba, Cd, Co, Fe, Cu, Mn, Mo, Ni, Pb, Sr, V, Zn, Si and Cr, 226Ra, 232Th and 40K. (El-Sayed *et al*., 2017). Siwa Oasis is eco-geographically isolated and is a nature reserve, so using of "Good Agricultural Practice" (GAP) is essential for agriculture production there such as using of various bio fertilization techniques (Hamed, 2018). Chicken eggshell (ES) is an aviculture by product that has been listed worldwide as one of the worst environmental problems, the chemical composition consists of calcium carbonate (94%), magnesium carbonate (1%), calcium phosphate (1%) and organic matter (4%) such as type X-collagen, sulfated polysaccharides and other proteins (Bashir and Manusamy , 2015). ES chemical composition and availability makes ES a potential source of filler for bulk quantity, inexpensive, lightweight and low load-bearing composite applications. There have been several attempts to use eggshell components for different applications; adding ES into food supplements for people and animals, art projects galore include egg shells as an ingredient, mosaics, paints, paper making, dying and carving (Yi *et al.,* 2004). The aim of this research work is to study the control of *S. littoralis* by using a new natural nanoparticles like bio-pesticide compounds such as *M. oleifera* leaf powder, boiled white and brown eggshells powder for chickens and kapritia spring water form Siwa Oasis.

Materials and methods

1.Laboratory studies:

1.1. Sample preparation:

1.1.1. Eggshells:

The samples of boiled chicken eggshellswhite and brown color was collected,then rinsed with clean water, then eggshells drying under hot sun for 3 days to dry then crushing themby mini food chopper or a coffee grinder to make a powder.

1.1.2. *Moringa oleifera* Lam. leaf powder:

Sample of leaves were obtained from farmer on Ismalia Governorate during summer season, then dried and grinding to powder.

1.1.3. Samples of kapritia Swia water

Samples of kapritia Swia water collected during early summer seasons 2018, used as it is without any additives then dipping the castoroil leaves on solution then dray under room temperature and put all leaves in glass gar contain 50 4th instar larvae of target pest

2. Insect culture:

The fourth instar larvae of cotton leafworm *S. littoralis* were obtained from laboratory colony, Cotton Leafworm Department, Plant Protection Research Institute, ARC, under laboratory conditions at 25 ± 1ºC and 65–70 ± 5 RH.

3. Bioassay of nanoparticle against 4th instar larve of *Spodoptera littoralis*:

3.1. Experiments were carried out under laboratory conditions, ten larvae of 4th instar of *S. littoralis* perreplicate for each treatment, it was obtained from culture reared on castor oil leaves for several generations.

3.2. Castor oil leaves were dipping on 50 ml of kaperitia water without any additives (absolute), then dry the water from the surface of leaves.

3.3. Estimated weight with 0.5 gm/leave of *M.oleifera* leaf powder used for fogging castor oil leaves for each replicate.

3.4. Eggshells powder white and brown used as nanoparticles by 0.5 gm/leave for each replicate and putted leaves as a food material on glass jars contain ten 4th instar larvae of *S. littoralis* for five replicates, then kept at

incubator at 25 ± 2° C and 60 – 65% RH., as shown in Figure (1) . All biological parameters of tested *S. littoralis* stages, fed on treated leaves such as larval duration, pupation, pupal duration,pupal weight, % moth emergence, sex-ratio, fecundity (eggs/ ♀), and hatchability %.

3.5. Statistical analysis:

Statistical analysis was carried out using Analysis of variance (ANOVA) was conducted on all data (SAS Institute, 1996).

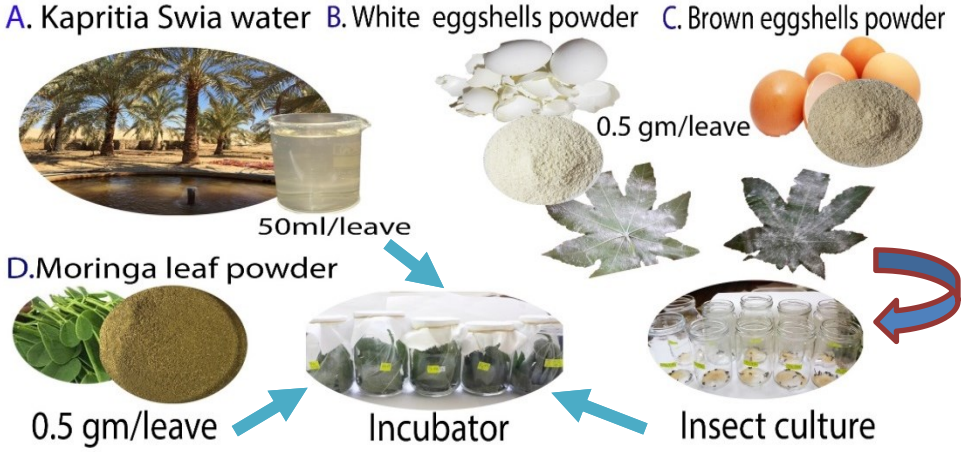


Figure (1): Novel natural bio-pesticide.

Results and discussion

1. Effect of nanoparticles material on the different biological aspects of the cotton leafworm *Spodoptera littoralis*:

1.1. Immature stages of *Spodoptera littoralis*:

The present results confirmed that, *M. oleifera* leaf powder, boiled white and brown eggshells powder for chickens and kapritia spring water form Siwa Oasis are considered a novel natural material such as green pesticide . Data as shown in Table (1) ,4th instar larval duration had impact after feeding on all treatments that caused larval mortality were 10, 8, 6 and 4%, respectively, compared with control 1%, pupal duration recorded highly significant between all treatments that caused highly reduction of pupation, it was recorded that 88% in case treatment kapritia water, while 90% after feeding on *M. oleifera* leaf powder, 92% brown eggshells powder and 96 % white eggshells powder. Both pupal weight of control and treated larvae on brown eggshells powder recorded no significant, while highly significant between other treatments. The maximam effect of kapritia water, *M. oleifera* leaf powder, brown and white eggshells powder on pupal stage induced 34, 26, 17 and 10% mortality, respectively, but 4 % only in control (Table, 1). Goswami *et al* . (2010) investigated that, the nanoparticles of SiO2 show nearly 100% mortality against *Sitophilus* oryzae L. (Coleoptera: Curculionidae). El-Sayed *et al*. (2017) recorded kapritia spring of Siwa Oasis content, major ions included chloride (Cl-)3702 mg/l, sulfate (SO42-)1800 mg/l, carbonate (CO32-), bicarbonate (HCO3-), sodium (Na+)1700(mg/l), potassium (K+), calcium (Ca2+) and magnesium (Mg2+590 mg/l)) and heavy metals included aluminum (Al), boron (B), barium (Ba), cadmium (Cd), cobalt (Co), iron (Fe), copper (Cu), manganese (Mn),molybdenum(Mo), nickel (Ni), lead (Pb), strontium (Sr), vanadium (V), zinc (Zn), silicon (Si) and chromium (Cr), that explain the highly mortality by treatment of kapritia water against cotton leafworm *S. littoralis.*

Table (1): Nanoparticles material affected on biological aspects of *Spodoptera littoralis* (immature stages) under laboratory conditions.

Biological aspect of 4th instar larvae of *Spodoptera littoralis*	Treatments					F.
	Kapritia Swia water	*Moringa oleifera* leaf powder	Brown eggshells powder	White eggshells Powder	Control	
Larval duration (days)	11±0.1 a	10 ±0.1 b	9.6 ±0.1 c	8.2±0.1 d	9.8 ±0.1 bc	75.08
% Larval mortality	6%	10%	8%	4%	1%	
Pupal duration(days)	12 ± 0.3 ab	13 ± 0.3 a	11.75± 0.2b	9.8 ± 0.1 c	9.5 ± 0.1 c	56.44
Pupal weight (mg)	326.05±11a	297.0±11b	260.6±7.7 c	291.09 ±5.1 b	244.0 ±8.1 c	11.48
% Pupation	88 %	90 %	92 %	96 %	99 %	
% Pupal mortality	34 %	26 %	17 %	10 %	4 %	

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

The present results coincide with Dimetry *et al.* (2017) mentioned that acceptability and antifeedant effect of *M. oleifera* leaves as host plant towards 1st and 4th larval instars of the cotton leafworm *S.littoralis* in comparison with castor oil leaves as a control and leaf extract as organic insecticide reported by (Ndubuaku *et al*., 2015).

1.2. Mature stages of *Spodoptera littoralis*:

Adult emergence percentage as illustrate on (Table, 2) low percentage had recoded 65,73, 82 and 89 %, respectively, in all treatments, on the other hand control was 95%. Results revealed that the highly percentage of adults malformed 62, 46, 39 and 21 % after treatment with brown eggshells powder, kapritia water, white eggshells powder and *M. oleifera* leaf powder, respectively. On the other hand, the treatments of different affected on pupal stages induced a high mortality, male and female malformed also that as shown in (Table, 2) compared with normal pupae on control treatment. The results consistent with **Adenekan (2019)** who recorded that, a significant difference in the eggs laid of bruchid beetles in cowpea seeds, the number of adults that emerged and lowest mean number of eggs of 6.40 was laid when 0.5 g *M. oleifera* flower powder was applied.

Table (2): Nanoparticles material affected on biological aspects of *Spodoptera littoralis* (mature stages) under laboratory conditions.

Biological aspect of 4th instar larvae of *Spodoptera littoralis*	Treatments					F.
	Kapritia Swia water	*Moringa oleifera* leaf powder	Brown eggshells powder	White eggshells powder	Control	
% Moth emergence	65 %	73 %	82 %	89 %	95%	
% Moth malformed	46 %	21 %	62 %	39 %	10 %	
% ♀ Moth malformed	23 %	12 %	19 %	4 %	5 %	
% ♂ Moth malformed	23 %	9 %	43 %	4 %	5 %	
Sex ratio (Female: male)	1:1	1:1	1:1	1:1.3	1:1.6	
Fecundity (Eggs / ♀)	850±183 bc	710 ± 154 c	1100 ±168.8 bc	1630 ± 155 ab	2230 ± 301.0 a	6.9

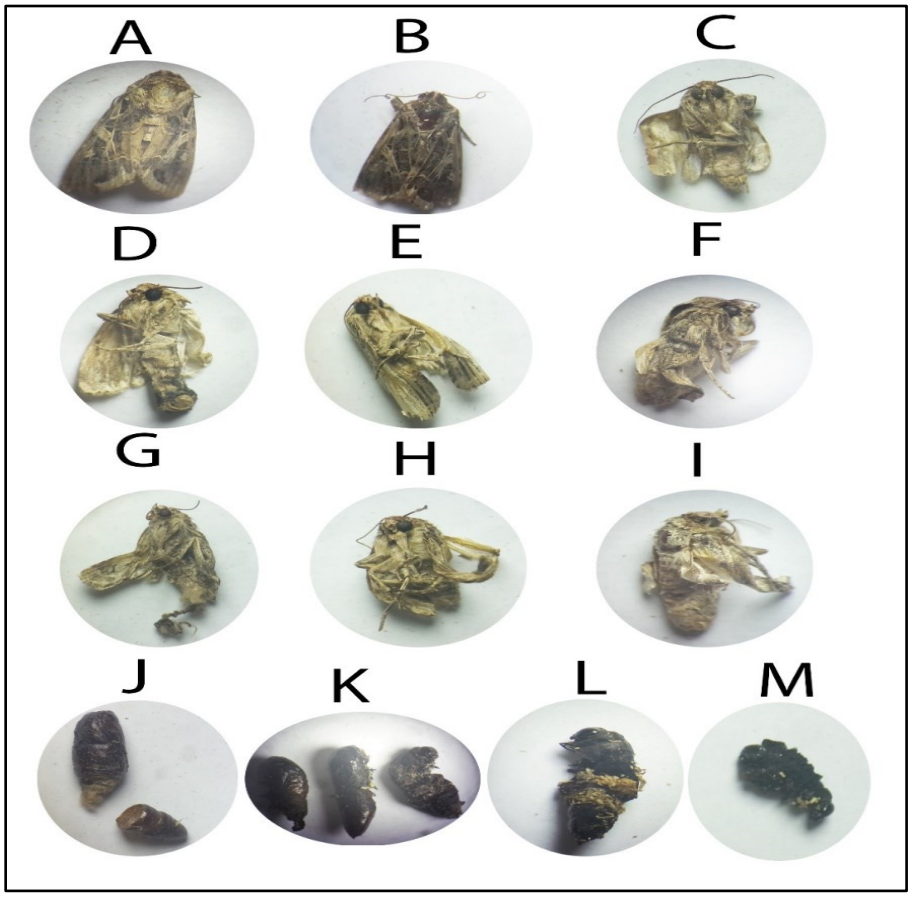
Means followed by different letters in each column are significantly different (P, 0.05).

As shown in (Table, 3 and Figures 2, 3 and 4) the different scores of malformed of the target pest after treatment with the new natural materials that highly affected so we need a lot of studies to improve this material for more efficiency and safety for used as a bio-insecticide to control insect pest and alternative the insecticides. This research work also will be helpful to use a new natural materials safe and useful on living organisms and safety on the environment and increase crop yield and sustainable agricultural.

Table (3): Scoring of larval – pupa – adult of *Spodoptera littoralis* after treated with nanoparticles material.

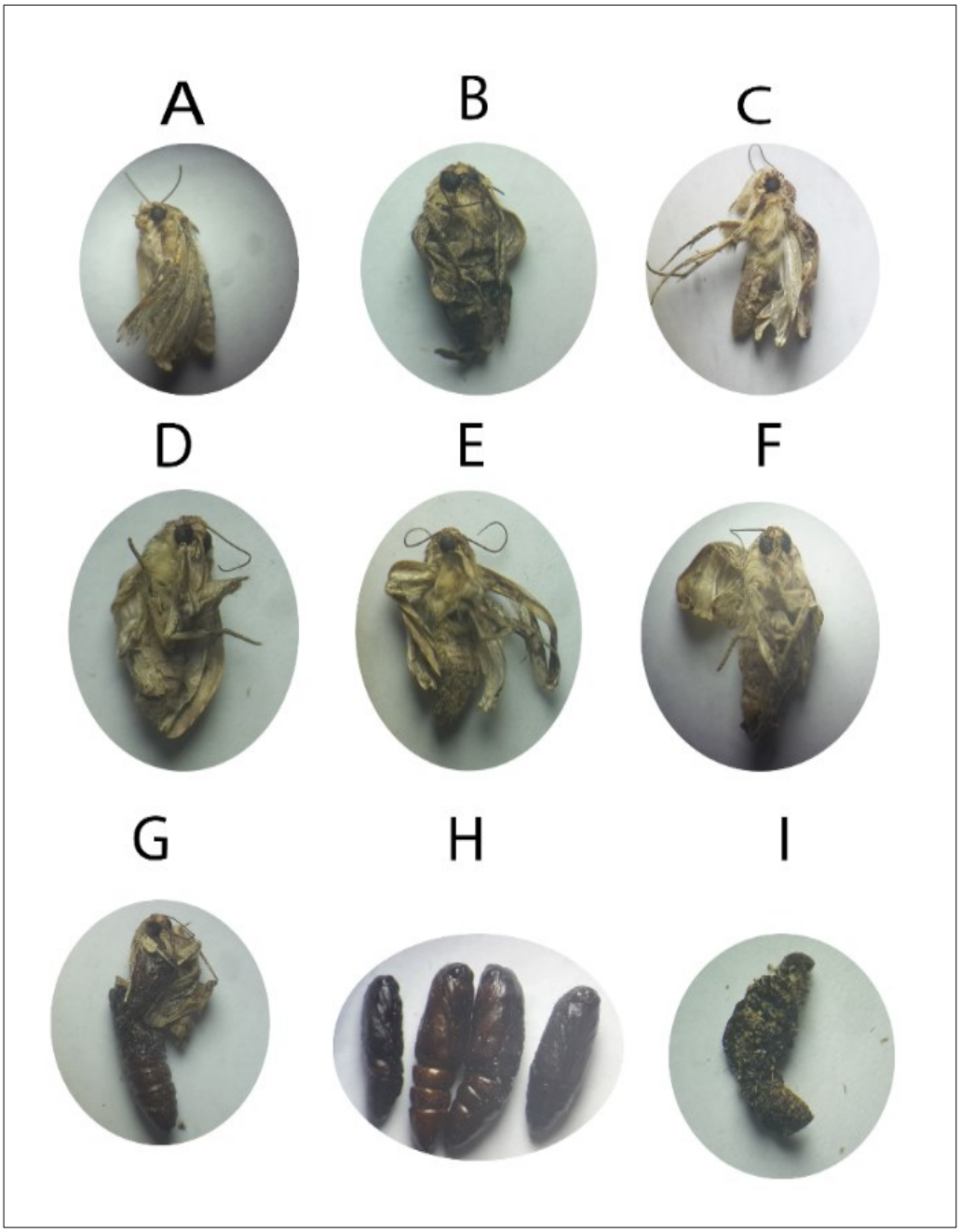
Scores	Characteristics	Kapritia Swia water	*Moringa oleifera* leaf powder	Brown eggshells powder	White eggshells powder
0	Adults seemed to be normal			+	+++++++
1	Adults with wings slightly curled	++++	++	+++++++	++
2	Adults wingless	+	++	+++++ +++++	++++++++
3	Adults severely curled	+++++++	++++++	+	
4	Adults attached with puprium	+	+	+	
5	Partial emergency (head and thorax)		+		
6	Partial emergency with head only		+		
7	Posteriorly partial emergency	+			+
8	Dead pupa	+++++ +++++ +++++	+++++ ++++	+++++ ++++	+++++
9	Larval pupal intermediate	+	++	+++	+
10	Dead larvae	++	++++	+	++

(+):Number of malformed adult moths



A: Normal Adults (Control), B: Adults seemed to be normal, C:Adults with wings slightly curled, D:Adults wingless, E:Adults severely curled, F:Adults attached with puprium. G: Adults wingless, H: Adults attached with puprium, I: Adults attached with puprium, J:Posteriorly partial emergency, K:Dead larvaeL:Larval pupal intermediate and M: Dead larvae.

Figure (2): Malformed of *Spodoptera littoralis* after treatments with kapritia Swia water.



A: Adults seemed to be normal, B: Adults with wings slightly curled, C:Adults severely curled, D:Adults severely curled, E:Adults wingless, F:Adults severely curled, G:Adults attached with puprium , H:Dead pupa and I:Larval pupal intermediate.

Figure (3): Malformed of *Spodoptera littoralis* after treatments with brown eggshells powder.

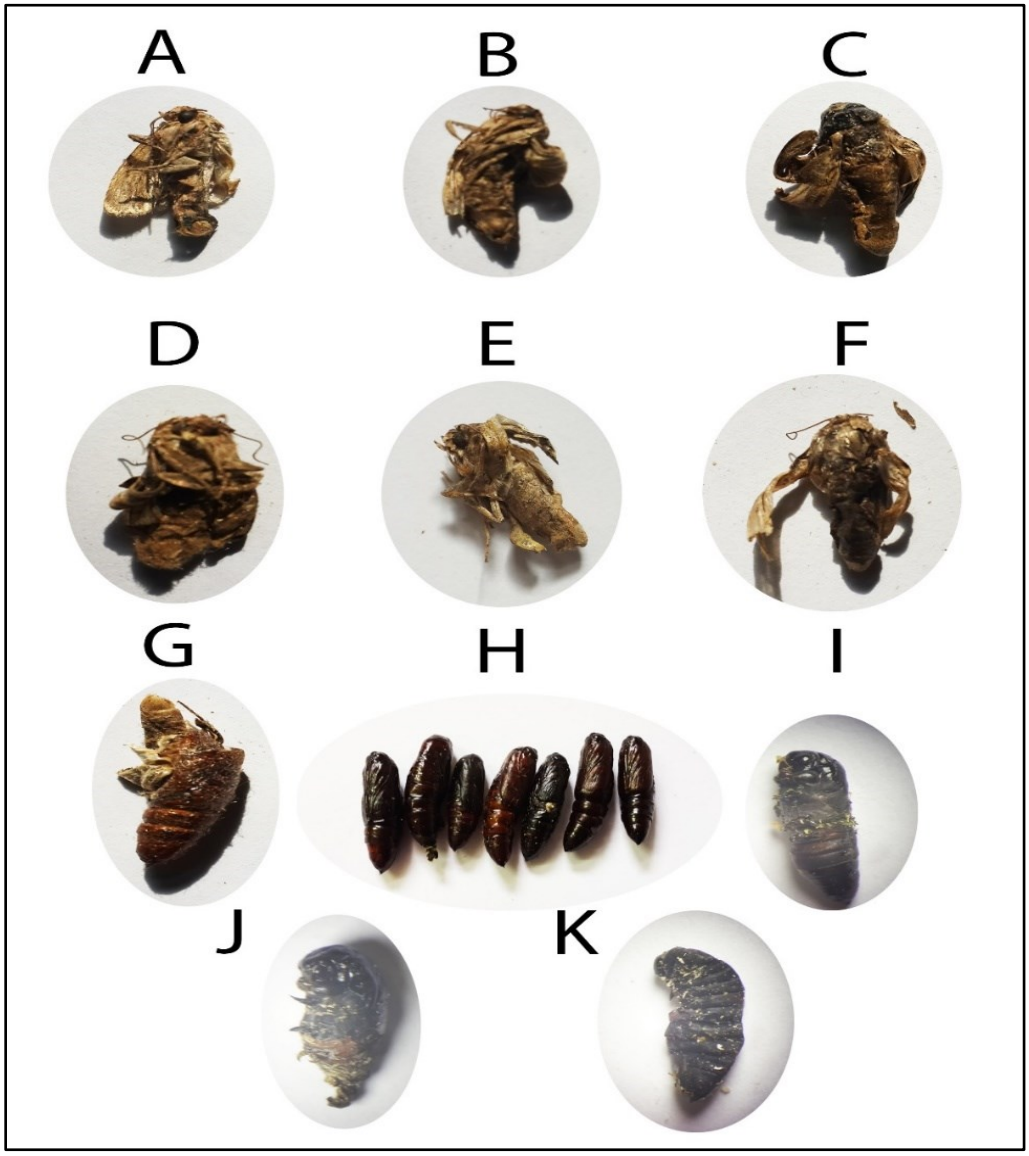


Figure (4): Malformed of *Spodoptera littoralis* after treatments with *Moringa oleifera* leaf powder.
A: Adults with wings slightly curled, B: Adults severely curled, C:Adults severely curled, D :dults severely curled , E:Adults wingless, F:Adults wingless, G:Adults attached with puprium, H: Dead pupa, I: Larval pupal intermediate, J: Larval pupal intermediate and K: Dead larvae.

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