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SYNTHESIS AND EFFICACY EVALUATION OF LEAD OXIDE NANOPARTICLE-BASED LARVICIDES: A NOVEL APPROACH TO MOSQUITO CONTROL AT KHAIRPUR MIR'S SINDH PAKISTAN

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ABSTRACT

Mosquitoes are the small flies belong to class insecta and order diptera. These are the life threatening organisms and kill almost one million people in a year by transmitting severe diseases, as it is the vector of many diseases. The life cycle of mosquito is divided in to four different stages which include egg, larva, pupa and adult. The larval stage of mosquito feeding is the most active stage. In this study the lead oxides nanoparticles were synthesized and used as insecticide on the mosquito larvae. The nanoparticles (NP) were prepared by adding lead acetate and NaOH. The solutions of lead acetate and NaOH were mixed thoroughly. The color of solution changed into cloudy. The mixture of solutions was heated till the color of solution turned into peach, then in orange color and finally turned into red. The water evaporated completely and ,the nanoparticles were washed several times to reduce pH until it reached around neutral. They were characterized by the scanning electron microscope. Five different dilutions of lead oxide nanoparticles were used on mosquito larvae which were arranged in five beakers. In each beaker 20 larvae were added. The concentrations of lead oxide NP were set of 2mg/L, 4mg/L, 6mg/L, and 8mg/L and10mg/L. The experiment was observed after 24 hours, 48hours and 72hours. It was observed that after 24 hours at the concentration of 2mg/L, 4mg/L and 6mg/L of PbO_3-NPs about 5% mosquitoes died. At higher concentration of PbO_3-NPs e.g. 8mg/L about 20% and at 10mg/L 30% mosquito larvae death was observed. After 48 hours the toxicity against larvae was increased with increase in concentration. At highest concentration of 10mg/L about 60% larval death was observed. After 72 hours the toxicity against mosquito larvae was higher than 1 and 2 days . At concentration of 6mg/L, 8mg/L and 10mg/L about 40%, 70% and 95% larval death was recorded.

INTRODUCTION

The word mosquito derived from the language of Spanish which means little flies (Brown and Lesley, 1993). Around 3500 species of mosquito are identified, The mosquitoes are classified into family Culicidea, order Diptera, class Insecta, of phylum Euarthropoda (Harbach and Ralph, 2008). The mosquitoes are life threatening organisms that kill almost one million people in a year by transmitting sever diseases (Poinar *et al*., 2000). Currently, there are 3000 mosquitoes species are present in the world that is grouped in the 39 genera and 139 sub genera (Clements, 1992; Reinert, 2000; Reinert, 2001).

About 226 million years ago mosquito departed from other insects. The fossils of mosquitoes found that are above 9 million years ancient. These fossils were same as the mosquito of modern century. Now a days in the world 3000 species of mosquitoes are present which are assembled in 39 genera and 135 sub-genera (Clements, 1992; Reinert, 2000; Reinert, 2001).

Introduction of Nanoparticles:

The term "Nano" is the Greek word which means "Dwarf". In technical language, meaning of "Nano" is 10-9 or one billionth of anything. Logically term nanotechnology is acquired for the nanometre size of particles (Bhattacharyyal et al., 2010). Nanoparticles can be found through some natural phenomenal activities like dramatic dust, volcanism, in weathering conditions and in the organic matter of soil after microbial action (Morales et al., 2017).

Nanoparticles of lead oxide:

*Lead oxide is a common term and group of inorganic compounds having different formulas as lead monoxide (PbO), lead tetroxide which is also called "red lead" (Pb3O4), and also in other colors that are black or gray oxides, is the combination of 70% lead mono oxide and 30% of metallic lead. The lead monoxide is also called the "litharge". The lead oxide is mainly used in the making of several instrumentation products. Because of its electrical properties it also used in the capacitors, silicon products, and electrophotography plates, ferromagnetic and ferroelectric materials. Furthermore it works as a rubber activator, curative proxy in elastomer, agent of sulfur remover in the manufacture of thioles and used in oil purifying and an oxidation catalyst in certain biological chemical processes. The litharge have many other significant applications in the manufacture of lead chemicals as desiccated colors, soaps and driers for paint and also used in the production of some lead salts and peculiarly worked in stabilizers for plastics (*Ritchie, 1970). The utilization of lead and the dispensation of lead oxide, associate with the various automobiles as it is the key element of automotive lead-acid batteries (*Sutherland et al., 2006).*

Material and methods:

1. Data Collection

For the collection of mosquitoes, larvae the sites were selected from Khairpur city region. The samples of stagnant water contained mosquitoes larvae were collected in many plastic jars. The larvae were brought to the department of Zoology of Shah Abdul Latif University Khairpur. By following the identification keys the species of Culex larvae were identified, identification was done by the help of compound microscope. After identifications, the species of Culex larvae were separated in the water beakers. After the separation of Culex larvae, the larvae were reared in the mosquito net. (Kamaraj *et al.*, 2009).

2. Mosquito rearing:

The identified Culex larvae were transferred into glass beakers for the purpose of rearing. The colony of larvae was maintained in the five beakers for different dilutions and the sixth was control. The glass beakers were also kept in the mosquito baby net for their protection. In each of six beakers twenty larvae were added. Each beaker was filled with the 250ml of tap water. The larvae were served with artificial food containing pinch of milk powder. The temperature and pH of each beaker were noted throughout the experiment.

3. Synthesis of lead oxide nanoparticles:

Molecular weight of lead acetate is 325.2g/mol.

4. Procedure:

To prepare lead oxide nanoparticles, first solution was prepared by adding 65.05g lead acetate in to 200mL of distilled water then mixed it properly. The second solution was created by accumulation of 80mg of NaOH into 200mL purified water and mixed it thoroughly.

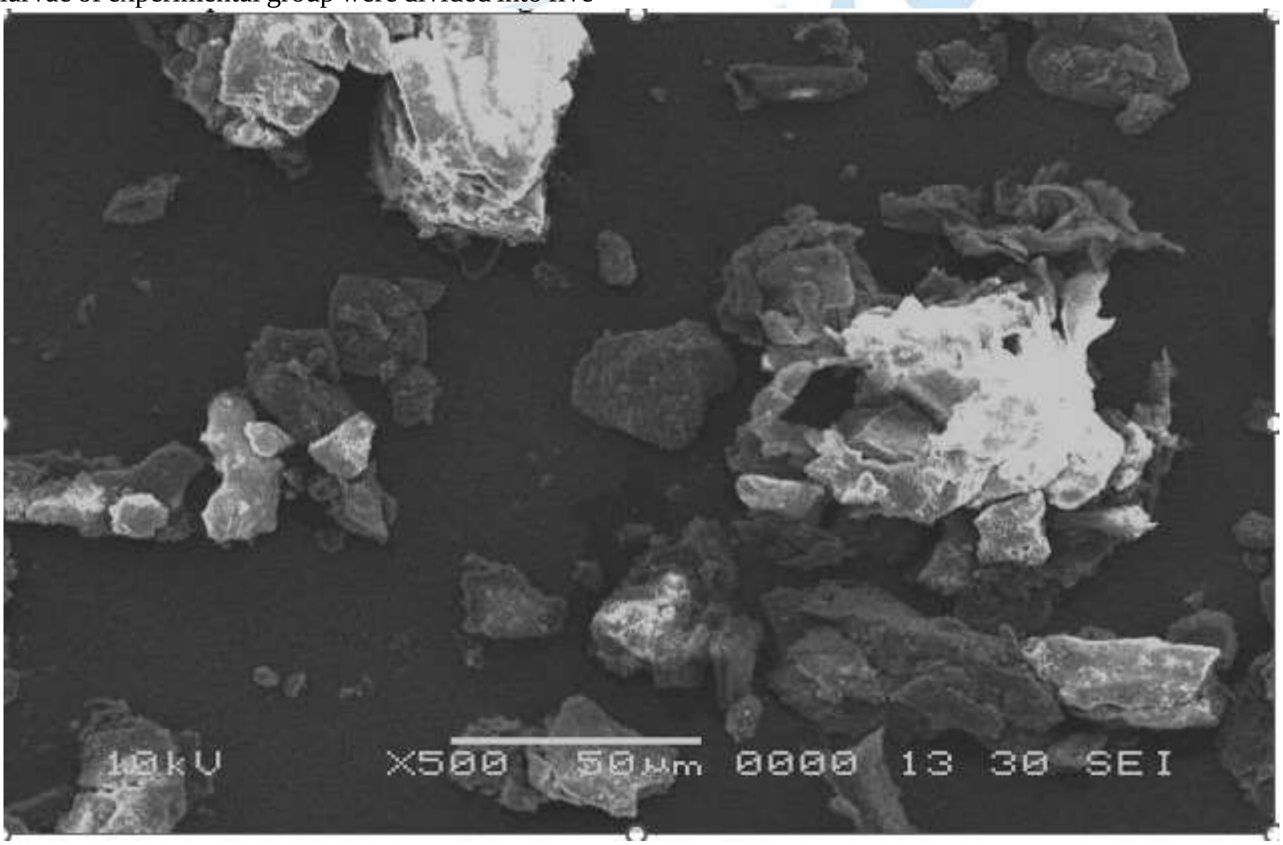
5. Working solution of lead oxides:

Added 60mL of lead acetate first solution into 50mL of NaOH second solution and mixed it thoroughly. The colour of solution changed into cloudy. The mixture of solutions was heated until the colour of solution turned into peach colour and then in deep orange color. After orange the colour of solution turned into red. The mixture was stirred continuously, until the color changes to red. The water of solution was removed through the process of evaporation; once the water was completely removed the process of heating was stopped. Nanoparticles were washed several times through the distilled water to reduce the pH to neutral. The nanoparticles were dried for overnight in the oven at 90 degrees (Amiri *et al*., 2016).

6. Characterization of lead oxide nanoparticles:
The characterization of nanoparticles of lead oxide was done with scanning electron microscope (SEM) to obtain the morphology and the size of lead oxide nanoparticles. The dehydrated samples were spread on the carbon tap on aluminum stub. Before the analysis, samples were coated with gold for better conductivity of electrons.

7. Experimental Design:
The larvae were divided and retained into two groups: control group and experimental group. The larvae of experimental group were divided into five beakers on the basis of different dilutions. The larvae of Culex were exposed to the lead oxide nanoparticles for three days. Then five different concentrations of 2mg/L, 4mg/L, 6mg/L, 8mg/L and 10mg/L were prepared from the working solution. The experiment was observed after 24hours, 48hours and 72hours of exposure and data was noted. The experimental group was matched to the control group. In control group 20 larvae were added and there were no any treatment was given to the control group (Marimuthu *et al*., 2010).

RESULTS AND DISCUSSION
Characterization
Scanning electron microscope (SEM) is the best technique for the confirmation of size and shape of lead oxide nanoparticles. The Scanning Electron Microscope images of pure bare lead oxide nanoparticles are shown in figure No:01.



However, we could not get the energy dispersive X-ray spectra of to approve the elemental configuration of obtained materials, due to the non-availability of liquid nitrogen and EDS detector to SEM. The resultant pure nanoparticles showed higher degree of agglomeration compared to already published oleic acid capped lead oxide nanoparticles. Synthesised nanoparticles are of spherical shape. The size of pure lead oxide nanoparticles is around 70 nm as already published studied using similar protocols. However, KT 2013 has reported the size of nanoparticles ranged 25 –

35 nm. The difference of size, in fact, in most cases is slightly higher than obtained with X-ray diffraction and dynamic light scattering analysis. The similar differences have already been reported in numerous published studies. AK 2013 showed selective area electron diffraction pattern (SAED) of lead oxide nanoparticles, having rings and spot pattern, which recommend the polycrystalline nature of lead oxide nanoparticles. Our data is in good agreement with the crystallinity index standards premeditated from XRD pattern.

Anti larvacidal Efficacy:

The growth and survival of *Plasmodium berghei anka* strain was found to sigmoidal. In the growth curve, most of larvae strains under normal conditions .The growth was slow in initial phase (day 0 day 3), followed by a sharp increase after day 5. Hence, the period (day 1 to day 3) within, where the growth of plasmodium was exponential as selected for inhibition assay. At this stage, most of parasites are amenable to physicochemical changes (Prescot 1993). Meanwhile at this period of growth, parasites provide a better clue of non-severe malaria in body.

Almost all larvae treated with lead oxide nanoparticles were killed after day 3. The percentage of killed larvae was dose and time dependent. This trend reflects the increased burden of nanoparticles interactions with culex larvae. Table 1 shows the dose-dependent effect of nanoparticles. The normal larvae were given water (as control) and nanoparticles were suspended in similar medium. In the lead oxide nanoparticles-treated group, the larvae mortality was at the initial value of 5% to maximum of 30% after day 0 to day 1 for the treatment, while for 2mg/L and 10mg/L, respectively. The larvae count was decreased from 10% to 85% after the day 1 treatment. The larvae count was remained almost in exponential phase in decreasing order until the day 3 treatments.

Table No: 1 Different dose of nanoparticles and their efficacy on mosquito larvae:

Dose	Subject	24h	48h	72h	Total mortality	Total viability
2mg/L	20	5%	5%	5%	15%	85%
4mg/L	20	5%	10%	15%	30%	70%
6mg/L	20	5%	15%	20%	40%	60%
8mg/L	20	15%	20%	25%	60%	40%
10mg/L	20	**30%**	25%	**35%**	**90%**	**10%**

Peak larvicidal effect was observed with 2mg/L of lead oxide nanoparticles by the day three from initial time. The survival rate then reduced 10% by day 3 with 10 mg treatments. In this time interval, percentage larvae survival in control medium retained steady up to day 3.

The dose-dependent larvicidal assessment can give a useful insight of mechanism of resistance of corresponding nanoparticle`s treatment. For example, for nanoparticles in a dose-dependent treatments could show positive and strong correlation, however for dose with inverse effect could reveal negative weak correlation as shown in table no: 02.

Table No: 02 the correlation between the activities of different dose of nanoparticles against larvae.

Covariance and Correlations of Parameter Estimates

		Dose	Natural Response
PROBIT	Dose	3.145	.523
	Natural Response	.074	.006

Covariances (below) and Correlations (above).

The time and dose-dependent activities of lead oxide nanoparticles (as shown in table no: 03) were significantly correlated ($p < 0.05$ for lower doses), as it is expected based on aggregation profile of nanoparticles (Figure 1). In contrast, it is interesting to note that no significant correlation is observed for higher dose treatments ($p > 0.05$). This further statistically confirms the nanoparticles effect was dose-dependent.

Table no: 03 it shows the parameters estimated

Parameter Estimates							
	Parameter	Estimate	Std. Error	Z	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
PROBIT[a]	Dose	3.745	1.774	2.112	.035	.269	7.221
	Intercept	-7.552	3.822	-1.976	.048	-11.374	-3.729

a. PROBIT model: PROBIT(p) = Intercept + BX (Covariates X are transformed using the base 2.718 logarithm.)

There was significant linear correlation in the results obtained for MLC_{50} ($R^2 = 0.96$, $n = 20$, $p < 0.001$), along slope and intercept (95% confidence interval) of each treated experiment (Table No: 04).

Table No: *04 the correlation between the larvicidal activities of different concentrations of lead oxide nanoparticles.*

Con.	Con. * 1	Log (Con.*1)	Treated	Observed response %	Linear response %	Linear probit
2	2	0.3010	60	18.333	11.2042	3.7840
4	4	0.6021	60	31.667	38.0221	4.6951
6	6	0.7782	60	43.333	59.0158	5.2279
8	8	0.9031	60	73.333	72.7676	5.6058
10	10	1.0000	60	93.333	81.5632	5.8990

The Figure (Chi-square test) for MLC50 interpretation for larvicidal application shows the correlation significance by the incubation time, dose-dependency at three days. The majority tested parameters produced different responses to each data point. This further reveals the increased MLC50 with reaction time. Such increase is might be due to reaction proceeding time, causing the alteration in regression of dose-dependent manner. In nut shell, there is significant differences of MLC 50 calculated from experimental data.

Table No: 05 Chi-square test applied nanoparticles to larvae

Chi-Square Tests				
		Chi-Square	df[b]	Sig.
PROBIT	Pearson Goodness-of-Fit Test	1.065	2	.587[a]

a. Since the significance level is greater than .150, no heterogeneity factor is used in the calculation of confidence limits.

b. Statistics based on individual cases differ from statistics based on aggregated cases.

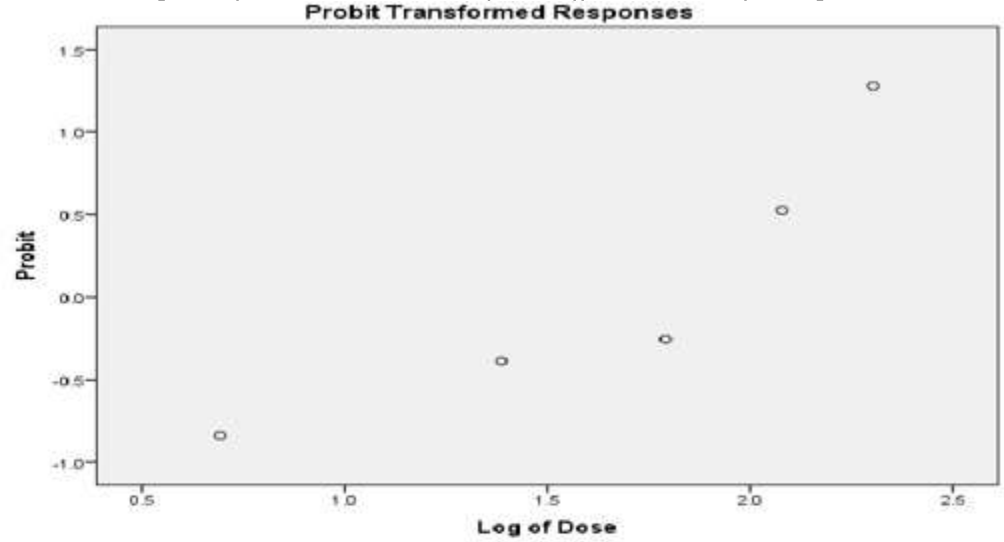
MLC50 values plotted against reaction time (Table No:05), the distributed points were in good agreement between the groups with aggregated doses of 2 mg/L, 4mg/L, 6mg/L, 8mg/L and 10mg/L, respectively. The Bland-Atman plot showed the significant estimation of MLC at increased doses and depicted the lowest reliable estimation of MLC50 > 5mg.

A single end point was used for simplicity and efficacy to interpret the data of all treated samples, particularly mortality measurement rather than multiple endpoint measurements as previously described (Wong Maker 1993a). In vivo, adopted larvae in three days of treatment were adequate for naked eye examination larvicidal evolution. At this point, the nanoparticles free samples showed visible survival of larvae, whereas, nanoparticles treated sample were visible to have dead larvae.

Table No: 06 correlations co-efficient for relation among the MLC 50 values.

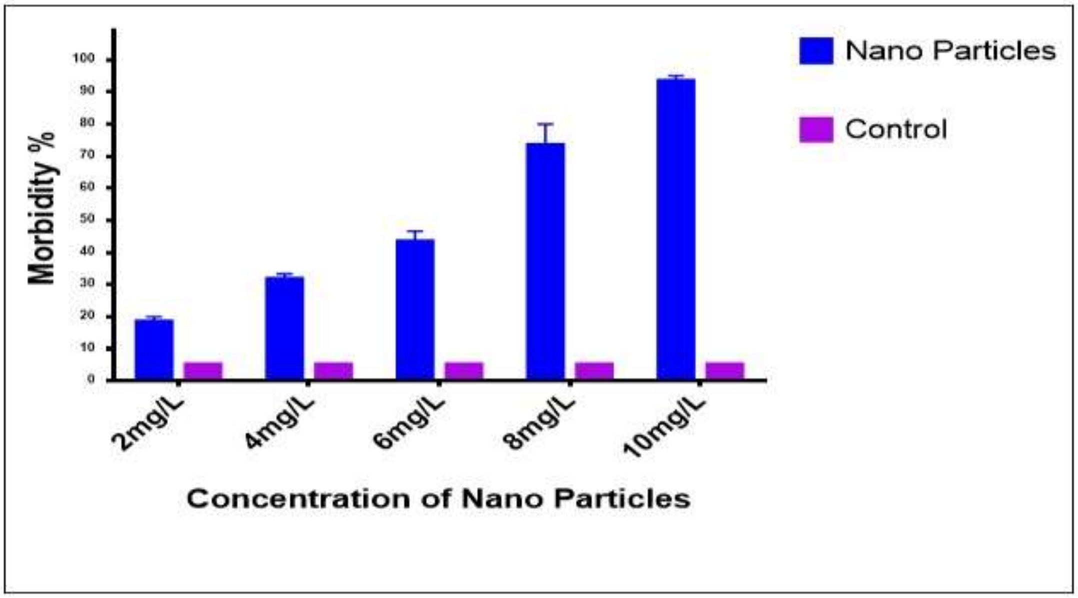
Cell Counts and Residuals							
	Number	Dose	Number of Subjects	Observed Responses	Expected Responses	Residual	Probability
PROBIT	1	.693	20	4	5.332	-1.332	.267
	2	1.386	20	7	5.466	1.534	.273
	3	1.792	20	8	8.269	-.269	.413
	4	2.079	20	14	14.038	-.038	.702
	5	2.303	20	18	17.921	.079	.896

***Table No: 07** the scatter pilot of MLC50 determined from different doses of nanoparticles.*



In vivo activities of larvicidal lead oxide nanoparticles are summarised in Table 08 and Table 09. It found in vivo morbidity threshold for lead oxide nanoparticles is based on response of culex larvae obtained from stagnant water. These results were in good agreement with results obtained with silver nanoparticles reported (Benelli 2018). The larvicidial materials have been employed in numerous studies using varieties of methods for in vitro and in vivo as drug effect assessment (Kalimuthu *et al*., 2017). However, there is close agreement between other nanoparticles and drugs with this study up to the given concentrations, where 2 mg to 10mg cut points were used with three days interval, exhibiting a MLC50 in between the cut points (Subramaniam *et al*., 2012). The in vitro larvicidal activities due to the novelty of this study for other larvicidal drugs and lead oxide nanoparticles could not be established through correlative in vivo studies.

Table No: 08 the morbidity level of mosquito larvae



Conclusion

In the present study, the lead oxide nanoparticles were synthesized and their efficacy was observed against the of mosquito larvae. Our results showed that lead oxide nanoparticles are effective against the larvae of mosquitoes in increased concentrations manner. The viability of mosquito larvae was decreased and the rate of mortality becomes high. Thus, this study suggests that the lead oxide nanoparticles are not effective at low doses compared to higher doses. Therefore, it is concluded that lead oxide nanoparticles have high potential against the mosquito larvae. Moreover, the environmental effects of using lead oxide nanoparticles as insecticides need further study.

REFERENCES

Brown, Lesley (1993). The New Shorter Oxford English Dictionary on Historical Principles. Oxford [Eng.]: Clarendon. ISBN 978-0-19-861271-1.

Harbach, Ralph (November 2, 2008). "Family Culicidae Meigen, 1818". Mosquito Taxonomic Inventory.

G. O. Poinar; et al. (2000). "Paleoculicis minutus (Diptera: Culicidae) n. gen., n. sp., from Cretaceous Canadian amber with a summary of described fossil mosquitoes" (PDF). Acta Geologica Hispanica. 35: 119–128.

Clements, A.N. 1992. The biology of mosquitoes, Vol.1. Development, nutrition and reproduction. Chapman and Hall, New York, NY.

Reinert, J.F. 2000. New classification for the composite genus Aedes (Diptera:Culicidae:Aedinii), elevation of subgenus Ochlerotatus to generic rank, reclassification of the other subgenera and notes on certain subspecies. J. Am. Mosq. Contr. Assoc. 16: 175-188.

Reinert, J.F. 2001. Revised list of abbreviations for genera and subgenera of Culicidae (Diptera) and notes on generic and subgeneric changes. J. Am. Mosq. Contr. Assoc. 17: 51-55.

C. Kamaraj&A. Bagavan&A. Abdul Rahuman&A. Abduz Zahir&G. Elango&G. Pandiyan. (2009). Larvicidal potential of medicinal plant extractsagainstAnopheles subpictusGrassi andCulextritaeniorhynchusGiles (Diptera: Culicidae). Parasitol Res. Vol and p104:1163–1171.

Bhattacharyyal A, A Bhaumik, P U Rani, S Mandal and T T. Epidi. 2010. Nanoparticles - A recent approach to insect pest control. Afr. J. Biotechnol., 9(24) : 3489-3493.

Morales-Díaz AB, Ortega-Ortíz H, Juárez-Maldonado A, CadenasPliego G, González-Morales S, Benavides-Mendoza A. 2017.Application of nanoelements in plant nutrition and its impact in ecosystems. Adv Nat Sci Nanosci Nanotechnol 8(1):013001.

Ritchie. E.J. Lead Oxides, Independent Battery Manufacturers Association, Inc., Largo, FL, 1974. W.E. Davis, Emissions Study Of Industrial Sources Of Lead Air Pollutants, 1970, EPA Contract No. 68-02-0271, W.E. Davis and Associates, Leawood, KS, April

Sutherland, Charles A.; Milner, Edward F.; Kerby, Robert C.; Teindl, Herbert; Melin, Albert; Bolt, Hermann M. 2006."Lead". Ullmann's Encyclopedia of Industrial Chemistry. Weinheim: Wiley-VCH. doi:10.1002/14356007.a15_193.pub2.

Asghar Amiri, Marziyeh Mohammadi, Marziyeh Shabani. 2016. Synthesis and Toxicity Evaluation of Lead Oxide (PbO) Nanoparticles in Rats. Electronic Journal of Biology. Vol.12(2): 110-114.

Sampath Marimuthu & Abdul Abdul Rahuman & Govindasamy Rajakumar & Thirunavukkarasu Santhoshkumar & Arivarasan Vishnu Kirthi & Chidambaram Jayaseelan & Asokan Bagavan & Abdul Abduz Zahir & Gandhi Elango & Chinnaperumal Kamaraj. 2010. Evaluation of green synthesized silver nanoparticles against parasites. Parasitol Res. Vol:108:1541–1549.

Benelli, G. (2018) Mode of action of nanoparticles against insects Environmental Science and Pollution Research 25:12329–12341.

Kalimuthu, K., Panneerselvam, C., Chou, C., Tseng, L.C., Murugan, K., Tsai, K.H., Alarfaj, A.A., Higuchi, A., Canale, A., Hwang, J.S., Benelli, G. (2017) Control of dengue and Zika virus vector Aedes aegyptiusing the predatory copepod Megacyclops formosanus: synergy with Hedychium coronarium-synthesized silver nanoparticles and related histological changes in targeted mosquitoes. Process Safetyand Environmental Protection. 109:82–96

Jayapal Subramaniam, Kalimuthu Kovendan,Palanisamy Mahesh Kumar,Kadarkarai Murugan ,William Walton. (2012). Mosquito larvicidal activity of Aloe vera (Family:Liliaceae) leaf extract and Bacillus sphaericus, against Chikungunya vector, Aedes aegypti. Saudi Journal of Biological Sciences. Vol: 19, 503–509.