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Effect of inorganic fertilizers on mortality and ovicidal action of dengue vector, *Aedes aegypti* L. (Diptera: Culicidae)

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Abstract
Present study was conducted to determine the effect of inorganic nitrogenous fertilizers like Urea, Muriate of Potash (MoP), Ammonium Sulphate (AM) and Complex on mortality and ovicidal action of *Aedes aegypti*. Each fertilizer was treated against third instar of *A. aegypti*. Ovicidal action and developmental duration of egg to larval stages along with physical parameters like pH, conductivity, total dissolved solid and salinity were recorded. The highest mortality of 62% was observed in Ammonium sulphate at concentration of 0.3% and 100% was observed at 0.5% concentration after 96 h of exposure. In the treatment of Complex, 100% larval mortality was observed at 0.5% concentration and next maximum of 36% larval mortality was recorded at a concentration of 0.6% after 96 h exposure. Analysis of variance, LC_{50} and LC_{90} and Tukey's HSD DMRT test revealed a significant impact of fertilizers on survival and development of *A. Aegypti*. Hence the use of inorganic products for the control of mosquitoes offers economically viable method.

Keywords: *Aedes aegypti*, inorganic fertilizers, mortality and ovicidal action.

1. Introduction

Millions of people suffer from insect-transmitted diseases. The primary vector of yellow fever, chikungunya, filariasis, schistosomiasis, dengue and severe dengue hemorrhagic fever is caused by the mosquito *Aedes aegypti* [1-3]. In Indian subcontinent, various species of Aedes, Anopheles, and Culex mosquitoes are important insect vectors of human diseases [4]. The estimate shows that more than 50 million people are at risk of dengue virus exposure worldwide. Malaria and other vector-borne diseases also contribute to the major diseases. Every year, there are 2 million infections, 5, 00,000 cases of dengue hemorrhagic and 12,000 deaths [5]. Mosquito borne diseases are endemic to more than 100 countries mainly in Africa, Americas, western Mediterranean, South and East Asia and West Pacific [6-8]. Vector-borne diseases are still major public health problems due to poor drainage system, especially during rainy seasons, the presence of fish ponds, irrigation ditches and the rice fields provide abundant mosquito breeding places [9].

The use of effective synthetic insecticides to achieve immediate results in the control of mosquitoes were increased however, their indiscriminate use of pesticide resulted in several problems, resistance and resurgence of pests [10-12]. Most of synthetic chemicals are expensive, destructive and toxic to humans, animals and other non-target organisms [13] Synthetic insecticides are more hazardous to handle, leave toxic residues in food products and not easily biodegradable [14]. Pesticide resistance are negative effects on non-target organisms, humans and the environment [15]. Mosquitoes develop genetic resistance to synthetic insecticides [16] and botanical pesticides are less toxic, delay the development of resistance and easily biodegradable [17] and to these problems have emphasized to develop new strategies for selective mosquito control [18].

Studies revealed that the mosquito breed in rice fields is not only due to availability of breeding sites which because of resource abundance such as high algal growth [19] due to the use of nitrogenous fertilizers in rice fields [20] and also breed in water containers, water storage jars, drums and tanks around houses[21]. Mosquitoes are known to breed from the rice fields, potentially in breeding grounds [22]. Aquatic invertebrates that inhabit the wetland ricefields are considered important as nutrient recyclers, rice pests, biological control agents, food items and vectors of human and animal diseases [23]. Ricefields and irrigation areas in tropical and subtropical regions create favourable habitats for the several other invertebrates vectors and intermediate hosts of human and animal diseases [24]. Intensive rice cultivation and introduction of high yielding rice varieties in the field has increased the use of nitrogenous fertilizers in the rice fields for more grain yield and resulting in more breeding of mosquitos [25]. Despite the important role of fertilizers in supporting high populations of mosquitoes, Victor and Reuben (2000) studied the effects of organic and inorganic fertilizers on mosquito populations in rice fields [26]

The biological and physico-chemical attributes of the aquatic environment alter adult vector capability [27]. Reisen *et al.* [28] reported that association between water quality and the vector ability. Larvae of mosquitoes in clear water of suitable pH, temperature and nutrient conditions have been found to thrive in aquatic bodies such as fresh composition.

Urea fertilized in wetland rice fields act as mosquito breeding stimulator, ultimately it generates controversies between agricultural and public health stakeholders in rice growing regions of the world [29]. The effective mosquito larval control requires ability to identify larval habitats [30-31]. The control method are aim to reducing mosquito breeding sites and biting activity by using a combination of chemical–biological methods and several advocated bio control methods to reduce the population of mosquito and to reduce the man and vector contact [32].

Therefore, in order to shed more light on the role of fertilizers as potential larvicidal activity as well as the increasing trend to use inorganic fertilizers to improve the crop and alternative methods to control the mosquito population, we conducted preliminary laboratory studies on four fertilizers formulation against *A. aegypti* larvae. Mortality and ovicidal action along with limited studies of physic-chemical characteristic were also carried out on the stability of dissolved formulation of aqueous solutions of the water at different concentrations

2. Materials and Methods

2.1. Culture Maintenance

The raft of *Aedes aegypti* eggs were collected from Centre for Research in Medical Entomology (CRME), Madurai. The mosquito larvae were reared in our laboratory at 35cm X 25cm plastic and enamel trays with tap water. The larvae were fed on dog biscuits and yeast powder at the 3:1 ratio. Mosquito larvae were maintained at laboratory temperature (27±3^{0}C) and 75-85% relative humidity with a photo period of 14±2 h light, 10±2 h dark.

2.2. Test for larvicidal activity

The agrochemicals which were used by the farmers in rice field like Urea (N-46%), Ammonium Sulphate (Nitrogen-20.6% and Sulphate- 23%), Complex (Ammonium-N-20%, Phosphate- 20% and Sulphate- 17%) and Muriate of Potash (MOP) (60%) were collected from retail shop at Alwarkurichi. Four different concentrations, 0.3, 0.4, 0.5 and 0.6 g/100 mL were prepared to each fertilizer with untreated fertilizer as a control.

The third instar of *A. aegypti* larvae was used to test the larvicidal activity of inorganic fertilizers like Urea, Muriate of Potash, Complex and Ammonium Sulphate. Different range of concentration of each extract was prepared to detect the mortalities. Ten III instar of *A. aegypti* were kept in 350 mL glass beaker containing 100mL of double distilled water and 0.3, 0.4, 0.5 and 0.6 g/100mL inorganic fertilizers. Five replicates for each concentration were maintained. A control set up with 100 mL of distilled water was also maintained. The percent mortality was recorded at the intervals of 24, 48, 72 and 96 h and their LC_{50}, LC_{90} at 95% confidence limits of lower (LCL) and upper (UCL) were calculated.

2.3. Physico- chemical analysis

The physico-chemical parameters of the various inorganic fertilizer treatments such as pH, hardness, salinity and total dissolved solid were tested by using PCSTestr 35 Multi-Parameter.

2.4. Ovicidal action

The fertilizers formulation diluted in the distilled water to maintain the mosquito larvae at various concentrations ranging from 0.3, 0.4, 0.5 and 0.6 g/100mL and untreated fertilizers were desired as a control. Eggs of *A. aegypti* mosquitoes were exposed to each concentration of fertilizers formulation. After treatment, the number of egg hatchability was counted. Each experiment was replicated for three times along with control. The hatch rates were assessed for 24, 48, 72 and 96h respectively, by following formula.

$$\text{Percent of egg mortality} = \frac{Total\ Number\ of\ hatched\ Larvae}{Total\ number\ of\ egg} \times 100$$

2.5. Total egg hatching duration

A.aegypti eggs were introduced in different concentration of inorganic fertilizers such as urea, muriate of potash, complex and ammonium sulphate at a concentration of 0.3, 0.4, 0.5 and 0.6 g/L. The time duration for hatching has been noted by visible larval count from the egg. Hatched and unhatched eggs were differentiated by microscopic observation.

2.6. Statistical Analysis

Profit analysis for calculating LC_{50} and LC_{90} at 95% confidence limits of upper and lower and chi-square values were calculated by using the statistical software, SPSS (21.0 ver.), significance level was set at *P<0.05*

3. Results

3.1. Test for Larvicidal activity

Larval mortality of *A. aegypti* after the treatment with various concentrations of inorganic fertilizers formulations like Urea, Muriate of Potash (MOP), Ammonium Sulphate (AS) and Complex were observed at the concentrationof 0.3, 0.4, 0.5 and 0.6 g/100mL. The results of III instar mortality of *A. aegypti* after the treatment were observed.

The result obtained from the inorganic fertilizers against mosquito, *A. aegypti* indicated that, among the four different fertilizers and concentration tested, Complex and Ammonium Sulphate were more effective against the target larvae (Table I). Both complex and sulphate offered 100% mortality at 0.5 and 0.6 g/100L during the period of 46 h and 72 h respectively. Adult mortality rates of 22 and 36% were recorded in Muriate of Potash (MOP) at 0.5 and 0.6 g/100mL concentration. Sixty two percent of mortality was noted at III instar by the treatment of Ammonium Sulphate at the concentration of 0.3 g/100mL and 100% mortality was observed after 96 h exposure. Totally, 56% of morality was observed at 0.4 g/100mL treated complex fertilizers after 72 h duration whereas no mortality was observed in urea treatment and control throughout the study period. There was no significant difference between the mortality rates of larvae exposed at various concentration of Urea.

The data of larvicidal activity of the inorganic fertilizers (Urea, Muriate of Potash, Complex and Ammonium Sulphate) dissolved water formulation against species of mosquitoes *A. aegypti* are presented in Table 2. In terms of lethal concentrations for 50% mortality after 72 h showed (LC_{50}) and (LC_{90}) inorganic formulation of fertilizers like potash, sulphate and complex are appeared to be most effective against *A. aegypti* larvae. Muriate of Potash showed (LC_{50}=0.720 g/100mL) and (LC_{90}= 1.210 g/100mL) followed by Ammonium Sulphate (LC_{50}=0.301 g/100mL) and (LC_{90}= 0.508 g/100mL) and Complex (LC_{50}=0.336 g/100mL) and (LC_{90}=0.462 g/100mL). Lethal concentrations for 50% mortality after 96 h of 95% of confidence level showed (LC_{50}) and (LC_{90}) inorganic formulation of fertilizers like Muriate of Potash, Ammonium Sulphate and Complex showed (LC_{50}=0.709 g/100mL) and (LC_{90}= 1.507 g/100mL) followed by Ammonium Sulphate (LC_{50}=0.264 g/100mL) and (LC_{90}= 0.407 g/100mL) and Complex (LC_{50}=0.313 g/100mL) and (LC_{90}=0.442 g/100mL). Whereas no LC_{50} and LC_{90} values recorded in control as well as and Urea treated owing to nil mortality.

3.2. Physico-chemical analysis

According to physico-chemical parameter of Urea showed there is no mortality from 24 h to 96 h of *A. aegypti*, there is a slightly change in the average pH, conductivity, Total dissolved solid and salinity compare to control as 5.5, 15.2, 10.9, 18.3 and 6.9 respectively. The larvae mortality decrease with increasing water pH, conductivity, Total dissolved solid and salinity. There is no change in the different concentration of inorganic formulation of urea. The Ammonium Sulphate, the larval mortality of *A. aegypti* was a petite increased with change the value of inorganic fertilizers formulation was decrease in pH, conductivity, Total dissolved solid and salinity like 4.1, 4.6, 3.3 and 3.1 respectively. In case the Complex and Ammonium Sulphate was 100% of mortality observed at the concentration of 0.5 and 0.6 g/L, whereas the average value of inorganic fertilizers showed lower than the urea and ammonium sulphate showed in fig 1.

3.3. Ovicidal activity

Table 3 shows the ovicidal activity of different inorganic fertilizer formulations against eggs of *A. aegypti*. The percent hatchability was inversely proportional to the concentration of extract. All the treatments of fertilizer formulations exhibited ovicidal activity against *A. aegypti* at all the concentrations, while maximum activity of ovicidal mortality was noted in complex 74, 82, 84 and 89 was noticed at 0.3, 0.4, 0.5 and 0.6 g/100mL respectively. The concentrations of complex fertilizer formulation were statistically significant, when compared to all other fertilizer formulation. Followed by, the fertilizer formulation Muriate of Potash showed the ovicidal activity of 62 at 0.6 g/100mL followed by Ammonium Sulphate formulation 52 and Urea 48 as compare to control whereas no ovicidal activity was observed. Lower ovicidal activity was observed in Urea (22.0.45) and Ammonium Sulphate (26.0.89) at the concentration of 0.3 g/100mL. There no significant egg mortality was noted in Urea and Ammonium Sulphate at all the concentrations except 0.4 g/100mL and all fertilizer formulation which was statistically significant when compare to control. Control eggs noted the 100% hatchability showed in table 3.

3.4. Total egg hatching duration

The results of the ovicidal bioassay are given in figure 2. Highest egg mortality rates of 48, 52, 62 and 86% were observed at the highest concentration (0.6 g/100mL) of Urea, Sulphate, Potash and Complex respectively. At 0.5 g/100mL concentration, Urea, Sulphate, Potash and Complex determined 38, 42, 54 and 84% of egg mortality, respectively. Lowest egg mortality rates of 22 and 26% were observed by Urea and Sulphate, respectively, at 0.3 g/100mL concentration. The development time of 1^{st} day egg of *A. aegypti* to 1^{st} instar stage differed significantly among the different fertilizer treatments. It took approximately six days for egg to 1^{st}instar in the control which was not significantly different. In the experimental treatment, it took 6-7 days longer than the control treatment for egg to larval development. The urea treated water concentration showed 2-5 longer days followed by potash 2-4 longer, complex showed 6-7 days and sulphate showed 5-7 days 0.3 g/100mL to 0.6 g/100mL respectively.

4. Discussion

Highest larval mortality was found in benzene extract of *E. coronaria* against the larvae of *A. stephensi*, *A. aegypti* and *Cx. quinquefasciatus* with the LC_{50} values of 79.08, 89.59 and 96.15 mg/L [33]. The laboratory test of fertilizer molinate at 1.0 ppm concentration cause 50% mortality and 2.4 ppm caused the mortality of 90% in *Cx. quinquefasciatus* [34]. Ammonium Sulphate showed 40% mortality rate and up to one week delay in development of larval time of *Cx. quinquefasciatus* [35].

There is no mosquito mortality was observed in various concentrations of urea formulation throughout the study period like as control. The same results were observed by Olayemi *et al.* [29]. Simpson and Roger [36] reported that NH4-N significantly enhanced dipteran populations [37]. This result is due to the breakdown of ammonium sulfate into NH_3^+ and SO4 $_2^+$ could negatively impact on mosquito. Ammonia is interrupt nervous transmission and this could result in high mortality rate [25]. Exposure of snail species in 1–1.25 g/L doses of Ammonium Sulphate for 48 h results in 100% mortality.

Higher levels of muriate of potash affect mosquito osmotic balance since K^+ and Cl^- ions are the primary constituents of larval malpighian tubules [38]. The larvicidal and ovicidal efficacies are governed by the type and concentration of different classes of insect growth regulators [39]. Aquatic invertebrates in the ricefields were affected by nitrogen fertilizer. Fertilizer effects on fauna were probably mediated through the photosynthetic aquatic biomass [40]. The deep placement of N fertilizer inhibited the development of dipteran larval population [41]. Phyto-extracts of *Pithecellobiumdulce* in hexane, benzene, chloroform ethyl acetate and methanol had significant larvicidal and ovicidal properties against filarial vector [42]. Govindarajan [43] reported larvicidal activity of crude extract of *Sida acuta* against *C. quinquefasciatus, A. aegypti* and *An. Stephensi* with LC_{50} values ranging between 38 to 48 mg/l.

Applied fertilizers may lead to increase in the number of salts in the water resulting into higher electro-conductivity, pH ranges between 6.5 to7 and high turbid water explained by the availability of food, suspended organic solids and to offer protection to the mosquito larvae from the predators are more favourable for mosquito breeding [44]. The present study supported by Pelizza *et al.* [45] and pH of neutral and near neutral pH 6.8 to 7.2 is preferable for breeding of many species of mosquitoes. At the value below 4.5 mortalities occur. The conductivity showed high in urea subsequently decreased in MOP, AM and Potash. These results increase the mortality of *A.aegypti*. David *et al.* [46] reported that *Anopheles*

mosquito prefer to breed in high electric conductivity.
Fertilizers are rich sources of inorganic compounds that can be widely used to develop growth and yield to get more income by farmer. Inorganic fertilizers are rectifying the nutritional defect in the land immediately. Laboratory studies reported that high concentration inorganic fertilizers are emerging as potential mosquito control agents. Our results showed that fertilizers formulation of urea, potash, complex and sulphate have significant larvicidal and ovicidal activity against *A. aegypti*. Chemical substance present in the leaf extracts may block the egg micropyle region, thereby preventing gaseous exchange and ultimately killing the embryo. Hoffmann and Lagueux [47] reported a role of ecdysteroid and juvenile hormone in the embryonic development of various insects. Insect growth regulators disrupt hormonal balance due to change in hormonal titer affecting normal embryonic development. Various chemical properties of the larval survival and ovicidal action related to pH, conductivity, Total Dissolved Solid and Salinity concentration of urea, potash, complex and sulphate affect larval development, survival and ovicidal action. The rice field fauna ostracod, dipteran larvae and oligochaetes respond positively to nitrogen fertilizer. Subramaniam *et al.* [48] reported that the LC_{50} and LC_{90} values of *A. vera* against first to fourth instars larvae were 162.74, 201.43, 253.30 and 300.05 ppm and the LC_{90} values of 442.98, 518.86, 563.18 and 612.96 ppm, respectively. Enhancement of the nitrifying and decomposing bacterial community by $(NH_4)_2SO_4$ additions also may have been responsible for the prolonged period of *Culex* in wetlands. Nitrogen based inorganic fertilizers may interrupt nervous transmission, which may be reflected as high mortality rate and prolonged larval development duration and total immature duration extended for six days at concentration of 10.0 g/L [49]. Neem coated urea gave 90% reduction in breeding of JE vectors [50].

Table 1: Larvicidal activity of inorganic fertilizers against III instars larvae, *Aedes aegypti*

Treatments	Concentration	% of Larval Mortality			
		24 h	**48 h**	**72 h**	**96 h**
Control	-	0.0±0.0^{a}	0.0±0.0 ^{a}	0.0±0.0 ^{a}	0.0±0.0 ^{a}
Urea	0.3	0.0±0.0 ^{a}	0.0±0.0 ^{a}	0.0±0.0 ^{a}	0.0±0.0 ^{a}
	0.4	0.0±0.0 ^{a}	0.0±0.0 ^{a}	0.0±0.0 ^{a}	0.0±0.0 ^{a}
	0.5	0.0±0.0 ^{a}	0.0±0.0 ^{a}	0.0±0.0 ^{a}	0.0±0.0 ^{a}
	0.6	0.0±0.0 ^{a}	0.0±0.0 ^{a}	0.0±0.0 ^{a}	0.0±0.0 ^{a}
Muriate of Potash	0.3	0.0±0.0 ^{a}	0.0±0.0 ^{a}	4.0±2.45^{ab}	6.0±2.45^{b}
	0.4	0.0±0.0^{a}	8.0±2.0^{b}	10.0±0.0^{bc}	14.0±2.45^{c}
	0.5	4.0±2.45^{a}	16.0±2.45^{b}	18.0±2.0^{b}	22.0±3.74^{b}
	0.6	18.0±2.0^{a}	28.0±2.0^{b}	30. ±0.0^{bc}	36.0±2.45^{c}
Ammonium Sulphate	0.3	0.0±0.0^{a}	26.0±2.45^{a}	46.0±2.45^{b}	62.0±3.74^{c}
	0.4	22.0±3.74^{a}	76.0±2.45^{b}	78.0±2.0^{c}	84.0±2.45^{d}
	0.5	24.0±4.0^{a}	78.0±3.74^{b}	84.0±2.45^{c}	100±0.0^{c}
	0.6	32.0±3.74^{a}	82.0±2.0^{b}	100±0.0^{b}	100±0.0^{b}
Complex	0.3	12.0±4.9^{a}	20.0±3.16^{b}	32.0±3.74^{c}	44.0±2.45^{d}
	0.4	14.0±2.45^{a}	44.0±2.45^{b}	56.0±2.45^{b}	66.0±2.45^{b}
	0.5	22.0±2.0^{a}	86.0±2.45^{b}	100±0.0^{b}	100±0.0^{c}
	0.6	24.0±2.45^{a}	100±0.0^{b}	100±0.0^{c}	100±0.0^{c}

Control- nil mortality; [a]The value represent Mean±SE of five replicates followed by the same letter do not differ significantly at (*P*=0.05) according to Turkey HSD test (DMRT).

Table 2: The LC_{50} and LC_{90} values different concentration of inorganic fertilizers against III instar larvae of *A. aegypti*

Fertilizers	72 h of 95% Confidence Limit		Chi-Square	96 h of 95% confidence Limit		Chi- Square
	LC_{50} (LEL& UFL)	**LC_{90} (LFL& UFL)**		**LC_{50} (LEL & UFL)**	**LC_{90} (LEL & UFL)**	
Urea	-	-	-	-	-	-
Muriateof Potash	0.720 (-)	1.210 (-)	0.358	0.709 (-)	1.507 (-)	0.462
Ammonium Sulphate	0.301 (0.97 & 0.368)	0.508 (0.417 & 1.459)	1.478	0.264 (0.007&0.327)	0.407 (0.330 &1.783)	1.340
Complex	0.336 (0.262&0.382)	0.462 (0.403 & 0.386)	2.321	0.313 (0.205& 0.361)	0.442 (0.382&0.721)	1.626

Table 3: Ovicidal activity of different concentration of In-organic fertilizers against *Aedes aegypti*

Treatments	Ovicidal action of *A. aegypti*[a]			
	0.3g/100ml	**0.4g/100ml**	**0.5g/100ml**	**0.6g/100ml**
Control	0.0±0.0^{a}	0.0±0.0^{a}	0.0±0.0^{a}	0.0±0.0^{a}
Urea	22±0.45^{b}	34±0.55^{b}	38±0.46^{b}	48±0.84^{b}
Ammonium Sulphate	26±0.89^{bc}	30±0.55^{c}	42±0.45^{b}	52±0.84^{b}
Muriate of Potash	34±0.55^{c}	52±0.45^{d}	54±0.55^{c}	62±0.45^{c}
Complex	74±0.89^{d}	82±0.45^{e}	84±0.55^{d}	86±0.89^{d}

[a]Values represent mean of five replicates: Mean±SD followed by the same letter do not differ significantly at (*P=0.05*) according to Turkey's HSD (DMRT)

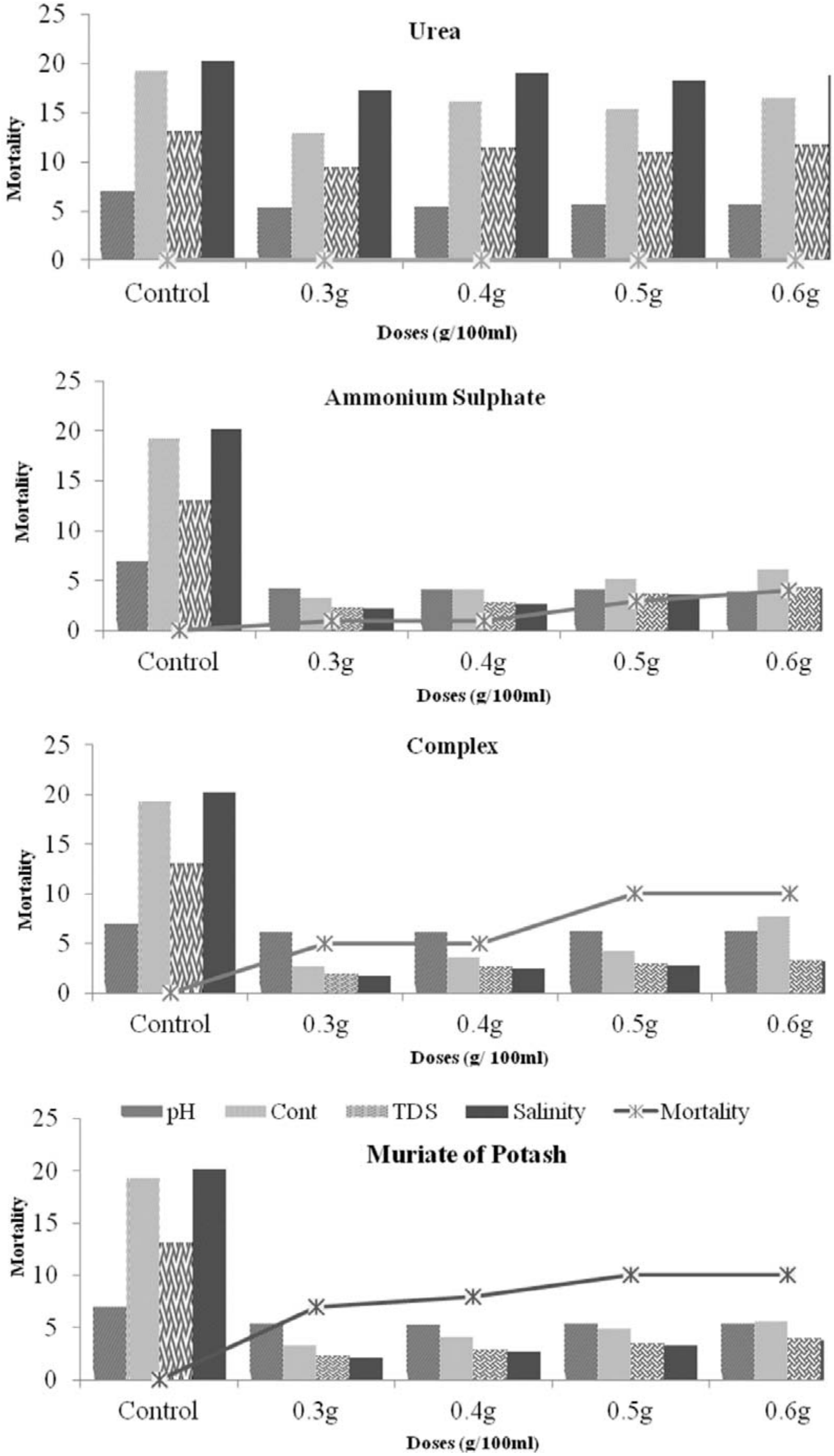

Fig 1: The physico-chemical parameter of fertilizers formulation against III instar larvae of *A. aegypti*

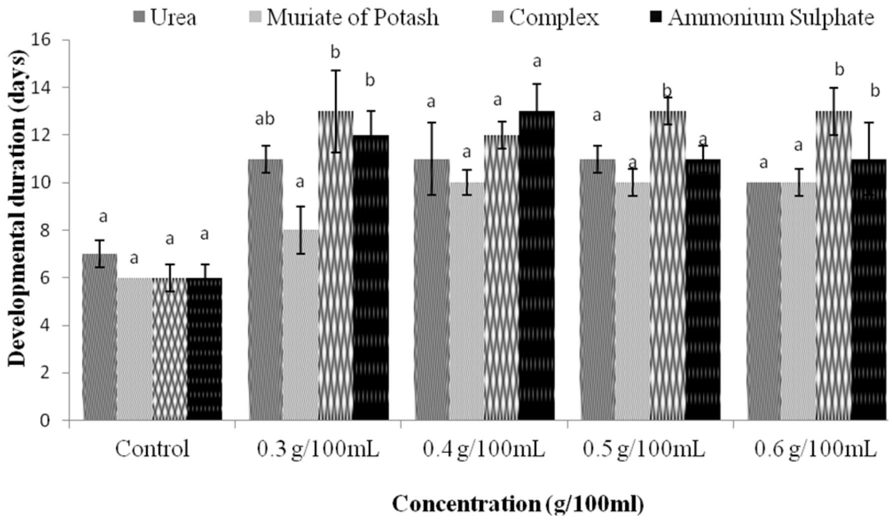

Fig 2: Effect of inorganic fertilizers applied to newly laid egg of *A. aegypti* on the duration of development time in days. Columns with same letters are not significant while those bearing different letters are significant at (*P=0.05*) according to Turkey's HSD (DMRT)

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