

Evaluation of (Fludora® Fusion) (A Mixture of Deltamethrin and Clothianidin) For Indoor Residual Spraying Against Insecticide Resistant Strain of Anopheles Stephensi in A Malarious Area in Bandar Abbas , Iran

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Abstract

Objetives: Malaria is a health problems in the world. WHO recomended several insecticides for Indoor residual spraying. Due to continious use of insecticides , the malaria vectors show resistant to different groups of insecticides. The aim of study was to evaluate the new mixture of pesticides Fludora® Fusion) (a mixture of deltamethrin and clothianidin) for indoor residual spraying against insecticide resistant strain of *Anopheles stephensi* in a malarious area in Bandar Abbas, Iran.

Materials and Methods: The residual effect of Fludora® fusion (WP-SB 562.5 g/kg in 100g wsb) was evaluated on various local surfaces of rooms such as mud and plaster as well as thatch roofs and wooden. Wordl Health Organization (WHO) standard cones using contact bioassays were carried out using laboratory reared, carbamate and pyrethroid resistant strain of *Anopheles stephensi* . Contact bioassays were carried out on sprayed surfaces. The cut of point of WHO is 80% mortality. Relative humidity and temperature of the test rooms has been recorded during the bioassay experiments

Results: Contact bioassay tests was carried out on days 1, 5,15,30, 60, 90, 120,150, 180,210,240,290 days after application . The mortality in all surfaces until 8 months is more than 80%. Mortality rate after 9 months on plaster and mud was more than 80%. The results form cement and wood was less than 80% .

Discussion and conclusión: Indoor residual spraying with Fludora® Fusion induced high and prolonged mortality of carbamate and pyrethroid-resistant malaria vectors for 9 months mostly due to the clothianidin component. This mixture is an important Integrated Vector Management for control of resistant malaria vector.

Introduction

According to the current WHO report (WHO, 2020) (1), malaria continues to have a devastating impact on people's health and livelihoods around the world. According to the latest available data, about 3.2 billion people were at risk of the disease in 97 countries, territories and areas in 2013, and an estimated 198 million cases occurred (range: 124 million–283 million). In the same year, the disease killed about 584 000 people, mostly children aged under 5 years in sub-Saharan Africa. In most countries where malaria is endemic, the disease disproportionately affects poor and disadvantaged people, who have limited access to health facilities and can barely afford the recommended treatment. Timely and affordable access to effective vector control interventions by populations that need them, including those to mitigate insecticide resistance and residual transmission, is a key component of malaria control and elimination efforts. Numerous interventions are under development aimed at addressing these areas. These include new insecticides, formulations or methods of application, new attractants and repellents, new bioactive agents (e.g., fungi or endo-symbionts), new mosquito life-cycle targets (e.g. sugar feeding, mating or oviposition phases), genetically modified mosquitoes, and endectocides.. WHO recommended insecticides for indoor residual spraying against malaria vectors are: DDT ,Malathion, Fenitrothion, Pirimiphos-methyl, Bendiocarb, Propoxur, Alpha-cypermethrin, Bifenthrin, Cyfluthrin, Deltamethrin

,Etofenprox, Lambda-cyhalothrin, Clothianidin (WHO, 2018) (2). Most of which were short-lived on home wall substrates (2–5 months) thus requiring multiple resource-demanding IRS campaign rounds when used in areas with stable malaria transmission. Malaria vectors have also developed resistance to these conventional insecticides which is now widespread and increasing in intensity across Africa [6] and this, together with their short residual effect is driving the development of a new generation of long-lasting IRS insecticides to which local vectors are largely susceptible. The neonicotinoid, clothianidin is a new repurposed insecticide which was recently added to the WHO's list of pre-qualified insecticides for use in indoor residual spraying [3]. Clothianidin presents a new mode of action which differs from that of conventional public health insecticides acting as an agonist on nicotinic acetylcholine receptors (nAChR) [4]. Owing to its novel mode of action, it shows potential to provide improved control of vector populations that have developed resistance to older public health insecticides. The addition of clothianidin to the portfolio of IRS insecticides also provides an opportunity to mitigate the development and spread of insecticide resistance in malaria vectors through the rotational use of IRS insecticides and the development of mixture IRS co-formulations [2]. Insecticide mixtures for IRS need to be explored with new public health insecticides when they become available because mixtures have the dual potential to improve malaria vector control through the combined effect of both active ingredients and contribute to insecticide resistance management, especially in areas where resistance to both active ingredients is not yet established [5]. In a previous experimental hut study in Benin, a tank mix of clothianidin and deltamethrin induced high and prolonged mortality (8–9 months) in wild pyrethroid resistant *An. gambiae* (*s.l.*) owing to the clothianidin component and early exiting of mosquitoes from experimental huts due to the pyrethroid component [6]. Currently, 8 registered neonicotinoid insecticides are available commercially worldwide [WHO 2020,b) (7)

Material And Methods

Study area; Malarious area of Bandar Abbas southern Iran was selected for experiments (Fig.1)

Materials:

The mixture of two insecticide, clothianidin and deltamethrin (Fludora® Fusion) is provided by Bayer company. Fludora® Fusion (WP-SB 562.5 g/kg) containing 500g/kg clothianidin (CTD) + 62.5 g/kg deltamethrin (DLT), in 100g water soluble bags.

Methods

Preparation of the different hut trial rooms with different surfaces

Initially, different hut rooms including cement, plaster, clay and wood surfaces selected in the appropriate village. These rooms are treated with insecticide while four untreated control containers have been maintained.

Residual spraying

Insecticide was sprayed using a compression sprayer recommended by WHO for the IRS which is equipped with a pressure gauge and HSS-8002 nozzles tips with regulator set at 24–55 PSI. Pesticide is dissolved in 10 liters of water in compression sprayer tanks. The sprayer discharge rate was set to 755 to 780 ml/min. The spray duration was adjusted to spray 19 m² in one minute. The operation is done by an expert under supervision

Mosquito species tested

Anopheles stephensi larvae have been collected from Hormoodar village (27°19'14.72"N, 56°19'14.80"E), in the south of Bandar Abbas city during and were transferred to the insectary. The larvae is reared into F1 generation for subsequent tests.

Adult susceptibility tests

According to the guideline developed by WHO, diagnostic dose of insecticides was used against female adult of *An. stephensi* mosquitoes. A total of four replicates were used as exposure (20-25 mosquitos per test, totally 100 specimens) for each insecticide and two replicates as control (totally 50 specimens). Test kits and insecticide-impregnated papers were purchased from the WHO collaborative center in University Sains Malaysia (USM), Penang, Malaysia. Bendiocarb 0.1%, permethrin 0.75% lambda-cyhalothrin 0.05 % and deltamethrin 0.05% were used against adult mosquitoes. The control test mosquitoes were exposed to papers impregnated only with the appropriate carrier oil for pyrethroids and carbamates that was without insecticide. Tests were performed on the F₁ progeny of wild-caught adult females of filed population, 3–5 days old fed with sugar. The mosquitoes were exposed to different insecticides by 60-minutes exposure time and 24 hours recovery period. A pad of a cotton wool soaked in 10% sugar solution was provided as feeding source of mosquitoes during the recovery period. Tests were carried out in an insectary maintained at (27±2)°C temperature and (75±10)% relative humidity, 14:10 light: dark¹. Mortality rate for each test was estimated at the end of tests. Results showed resistance to both pyrethroid and carbamate insecticides with mortality rates of (84.31±2.62)%, (89.66±2.34)%, (82.27±5.20)% and (86.10±3.61)% to lambda-cyhalothrin, deltamethrin, permethrin and bendiocarb, respectively. *An. stephensi* showed resistance to both pyrethroid and carbamate insecticide groups that used in this study (lambda-cyhalothrin, deltamethrin, permethrin, and bendiocarb).

Bioassay tests

The bioassay tests were carried out for evaluation of residual effect of insecticide by WHO cones. The cones was fitted on different treated surfaces using rubber band. About 10–12 sugar-fed, 3-5 days old female mosquitoes was gently released into each cone at the vertical position. The mosquitoes have been exposed for 30 minutes to each treated surfaces in five different replicates. The same procedures were carried out for control container. At the end of exposure time, the adults transferred into clean cups with cotton wool pad containing 10% sucrose solution and were kept in the insectary for 24h recovery period, the time for recording the mortality rate. The mortality followed after 48 and 72 hours as well (WHO, 2020). (Figure. 2)

Application method:

Minimum safety instructions and protective measures must be observed at all times. The operators will ensure that the insecticide formulation is safely and correctly applied. Standard Operating Procedures (SOPs) as well as WHO manual prepared for field staff working in national vector control programmes (WHO, 2003) applicable for Phase II trials must be available on site (e.g. safe use of compression sprayers, handling and spraying techniques, protective clothing and visors). The hut walls will be sprayed to attain dosages as per manufacturer's recommendation. The spray men will be carefully supervised during spraying. Products will be applied via Indoor Residual Spraying (IRS) with the maximum application rate amounting to 40 (50) mL spray/m². Only one round of spraying will be done. Calibration of the spray pumps will be done to obtain uniform and good quality spraying for the targeted dose. Protective clothing, goggles, gloves, etc. are provided to all spray men for their general safety.

Sachets and waste water will be disposed of according to WHO guidelines (WHO, 2007).

Statistical analysis

Data obtained from different replicates were collected for each surface. The mortality rate under 80% is considered as threshold level. Tests with control mortality rate between 5 and 20%, were corrected using Abbott's formula.

Calculation of mortality rate

Mortality rate in plaster, mud cement, wood surfaces has been calculated and plot the diagram.

Application equipment and other materials

One Hudson® Expert compression sprayer fitted with a constant flow valve (CFV), Safety equipment: overalls, boots, helmet, visor, gloves, chemical mask. WHO testing cones (complete kit with cones and aspirators), Consumables including: cotton wool, paper cups, marker pen, masking tape, gloves, glucose, elastic bands, netting material, shoe tacks, tetramin, cerelac, filter papers, pipettes (with rubber teat), distilled water, 30cm by 30cm by 30cm mosquito cages, mouth aspirators, stop watch, weather station (thermometers, hygrometer, etc.), tiny tags/labels, mosquitoes (charged per organism from the insectary), cool boxes and equipment to transport mosquitoes to insectary, cement, whitewash, sand, plywood/ceiling board, grass thatch, sheets galvanized iron, mud daub, insecticides, nails.

Results

Result of contact bioassay. The results of bioassay test on different surfaces are illustrated in Figures 3-6.

Contact bioassay tests was carried out on days 1, 5, 15, 30, 60, 90, 120, 150, 180, 210, 240, 290 days after application. The mortality in all surfaces until 8 months is more than 80%. Mortality rate after 9 months

on plaster and mud was more than 80%. The results from cement and wood was less than 80% . The cut of point of WHO is 80% mortality. Relative humidity and temperature of the test rooms has been recorded during the bioassay experiments.

Discussion And Conclusion:

Resistance to different insecticide classes such as pyrethroids, organophosphate, organochlorine, carbamates were evaluated using WHO guidelines. Results showed a wide variety of susceptibility/resistance status to these chemicals according to the location, historical context of pesticide used, genetic background of vectors, age and abdominal conditions of adults may play a role in the susceptibility status of these species to different insecticides. Fludora® Fusion has been developed specifically for vector control applications; it is the first product intended for indoor residual spraying campaigns which combines two unrelated modes of action, providing optimum effectiveness under conditions of insecticide resistance. The dual mode of action provides more robust and consistent residual activity compared to either active ingredient applied alone. The presence of a second mode of action helps to slow down the potential development of resistance to clothianidin. Fludora® Fusion and clothianidin WG 70 showed mortality rates over 80% WHO bio-efficacy threshold on cement walls either with susceptible or resistant *An. gambiae* s.s. over a period of 10 and 9 months, respectively. Treatment with Fludora ® Fusion and clothianidin WG 70 on the mud walls showed residual effect for 6 months and 5 months respectively against both susceptible and resistant mosquitoes (8).Resistance of *Anopheles gambiae* to the new insecticide clothianidin associated with unrestricted use of agricultural neonicotinoids in Yaoundé, Cameroon has been reported (9).

Declarations

Ethics approval and consent to participate: Applicable

Consent for publication: Applicable

Availability of data and material: Applicable

Competing interests: Not applicable

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Authors' contributions: All authors were involved

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Figures



Figure 1

Map of study area, Bandar Abbas. Hormozgan Province, Southern Iran



Figure 2

Evaluation of (Fludora® Fusion) on different surfaces

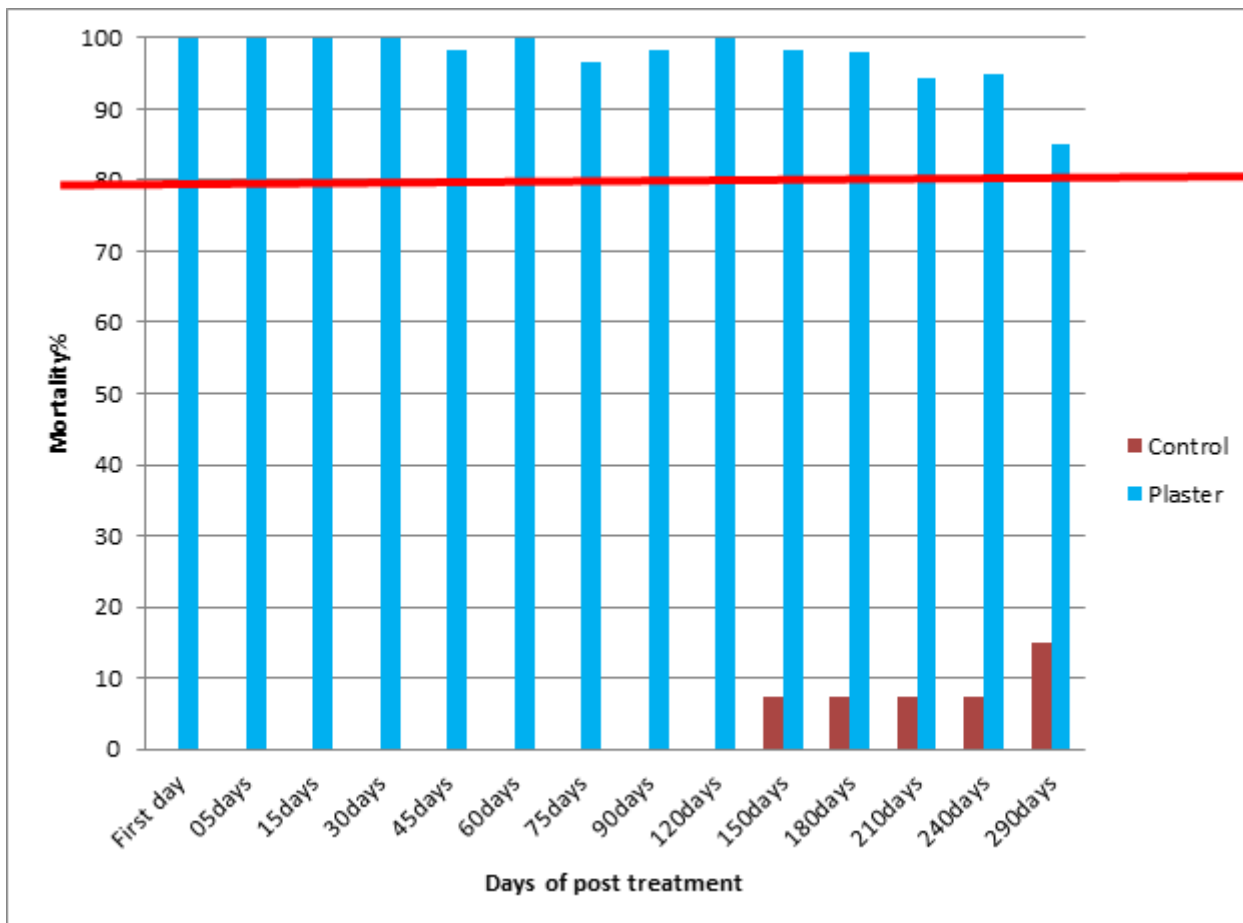


Figure 3

Residual effect of (Fludora® Fusion) on plaster surfaces

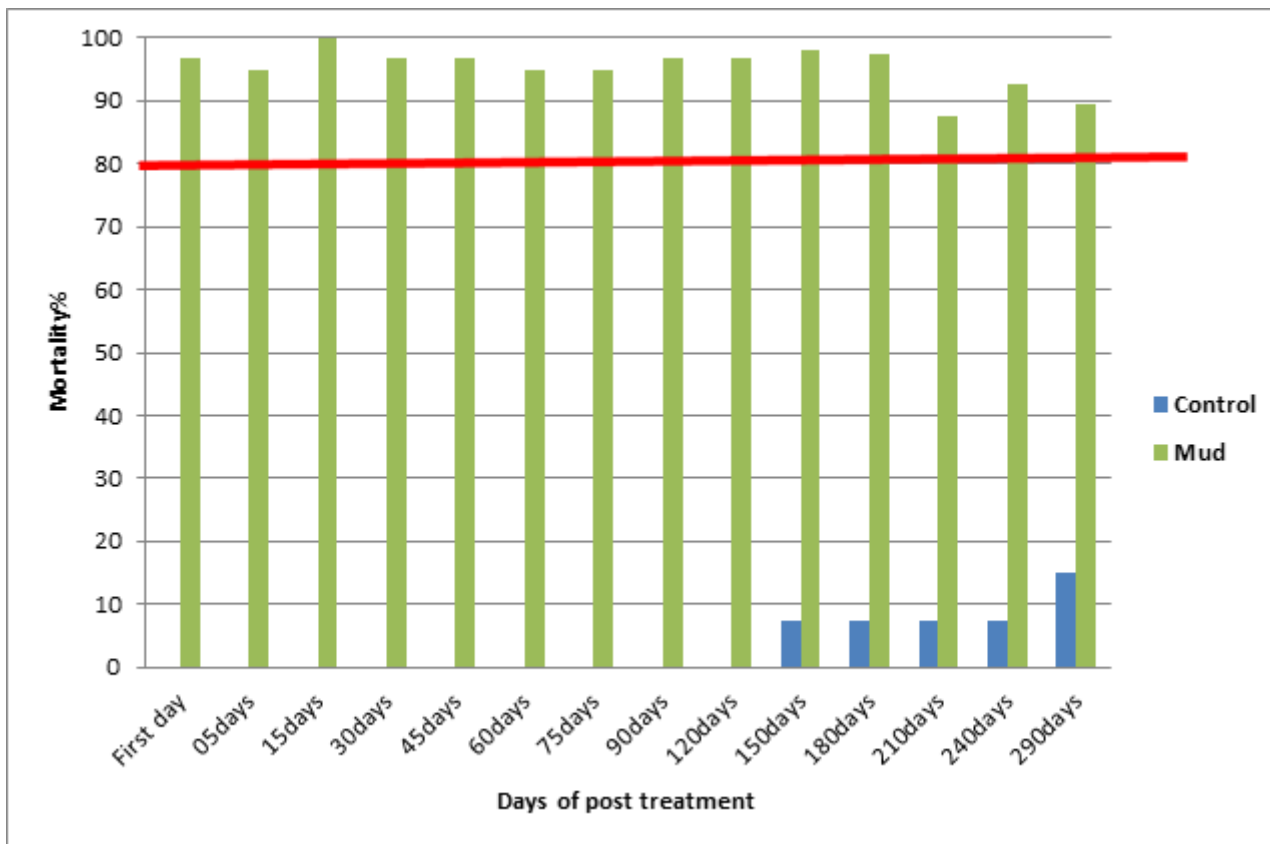


Figure 4

Residual effect of (Fludora® Fusion) on mud surfaces

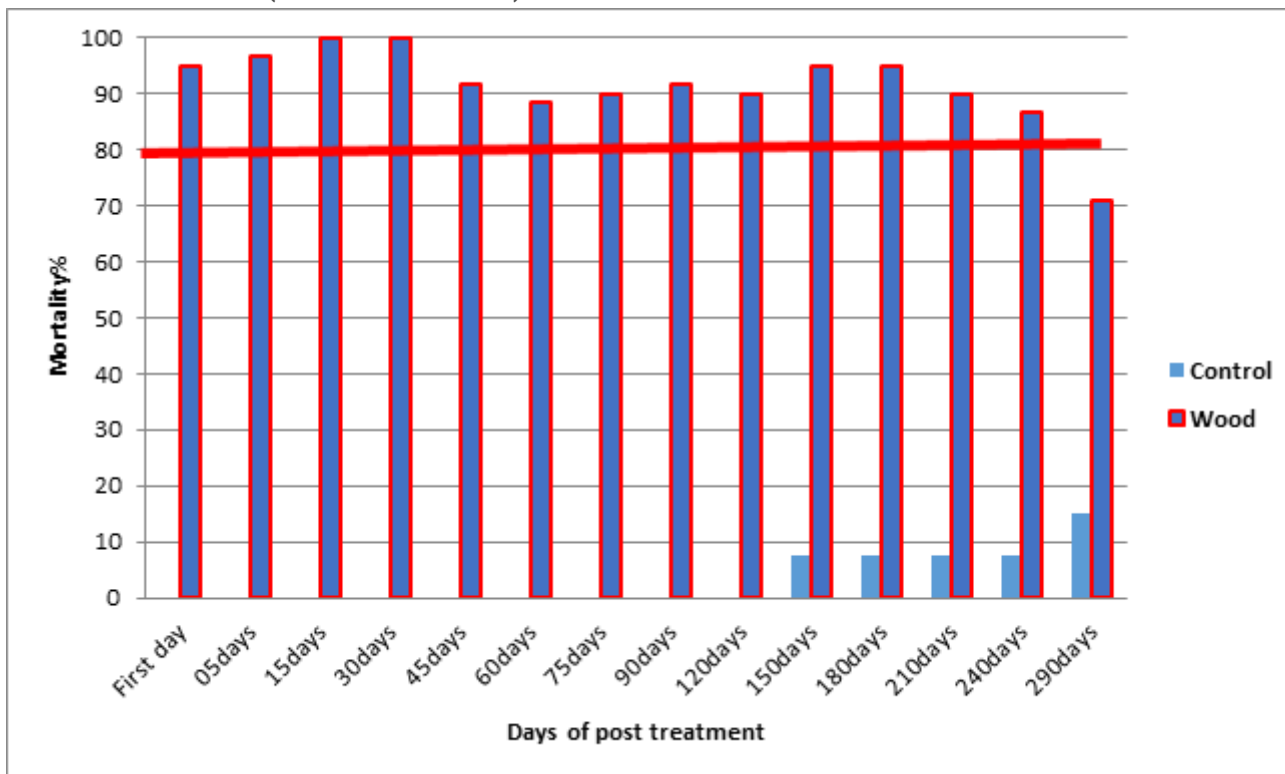


Figure 5

Residual effect of (Fludora® Fusion) on wood surfaces

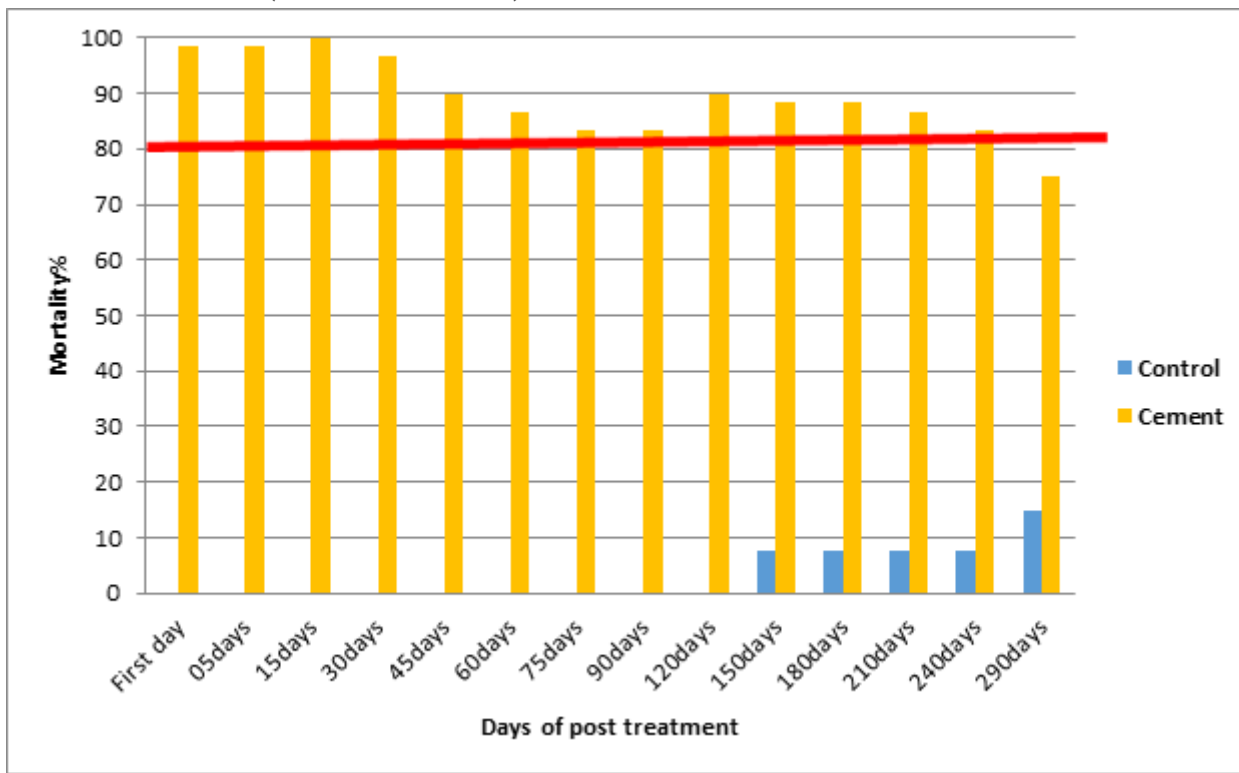


Figure 6

Residual effect of (Fludora® Fusion) in cement surfaces