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The Effect of some Protozoacides, on the Survival of Symbiotic Flagellates of *Coptotermes heimi* and *Heterotermes indicola*.

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Abstract

Coptotermes heimi and *Heterotermes indicola* are lower termites which harbor protists flagellates for the digestion of lignocelluloses, a main component of wood. In the present work effect of anti-protozoan chemicals, sulfaquinoxaline, sulfadimidine, diaviridine, entox and resochin was observed on the workers and soldiers of the two selected termite species and their gut flagellates. Filter papers were dipped in 10ml solution of 2% anti-protozoan chemicals, dried at room temperature and termites were forced to feed on these filter papers placed in the petriplates and maintained at 25°C. There was a gradual and significant decrease in flagellate's population existing in the hind gut of termites during 72 hours of feeding on the drug impregnated filter papers followed by the death of workers in each case except entox. In the case of soldiers no significant difference in mortality was observed in termites and their gut flagillates compared to the control including entox. Anti-protozoan drugs used in this experiment, other than entox, could be the best replacement of insecticides for the termite control.

Keywords: *Coptotermes heimi;* sulfaquinoxaline; sulfadimidine; diaviridine.

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1. Introduction

Coptotermes heimi and *Heterotermes indicola* (Isoptera: Rhinotermitidae) are lower termites which harbor protists flagellates and bacteria for the digestion of cellulose a main component of wood [1–3].

The contribution of bacteria in producing cellulases is considerably less as compared to that produced by flagellates [4]. Therefore, it seems logical to consider that any harm to the entozoic flagellates would produce a fatal effect on their host termite. However, the higher termites which are flagellates free contribute the 75% of all termite species and rely upon the cellulases secreted from their own gut wall [3]. In the present study the protozoacides (sulfaquinoxaline, sulfadimidine diaviridine, resochin and entox) were selected to study effects on termites and their gut flagellates. The sulfonamides (''sulfas'') had been discovered in the 1930s, as antibacterial mediators but later on their effectiveness had been shown to include eukaryotes[5].The antiprotozoal activity of sulphonamides, is due to the competitive inhibition of folic acid synthesis. The biosafety of the drug derives from the fact that mammals and birds ingest folic acid in their diet [6,7]. Resochin (chloroquinine) was discovered 75 years ago and is used in many regions of the world as a reliable treatment against simpler forms of malaria [8]. The quinine another anti-malarial drug extracted from *Diospyros sylvatica* roots, showed termiticidal activity on exposure to *Odontotermes obesus* [9]. In the light of the information and biosafety of the sulfadrugs and resochin, these drugs were tried to kill symbiotic protozoan residing in the gut of termites. Now days insecticides are in use to control termite infestations, but their use kills beneficial organisms thereby disturbing the ecological balance and these can accumulate in soil vegetables and fruits leading to medical problems, and lastly, in many countries, there is a ban on the use of many pesticides[10]. In the light of the activities of sulfonamides and resochin against a variety of pathogens, in the present investigations, we used these against the entozoic flagellates of termites.

2. Materials and methods

Termite species "*Coptotermes heimi* and *Heterotermes indicola*" were collected from mulberry trees found in the grounds of government college university Lahore Pakistan. The anti-protozoan drugs, Entox, resochin, Sulfaquinoxaline (SQ), Sulfadimidine (SDM) and Diaveridine (DV), tried to determine their action on the protozoan fauna of two species of lower termites i.e. *C. heimi and H. indicola.* Solution of each drug (2 %) was prepared in distilled water and filtered. The filtrates contents were used for the experiment. The Whatman filter papers were impregnated with 10 ml aqueous solution of each drug and kept in separate petridishes and air dried. Hundred termite workers were placed in each Petri dish carefully over drug-impregnated filter paper. In order to maintain humidity the filter papers were kept moistened by pouring 3ml of distilled water on them, after each 24hr of the experiment. The experiment was repeated taking termite soldiers instead of workers and a control was run in which the filter papers used were soaked in distilled water only. Percentage mortalities of both termite workers and soldiers were counted physically after each 24hr for 72hr. The same experiment was repeated but this time five-termite workers and soldiers were dissected from each set of

experiment, in 2% saline solution after each 24hr to study their flagellate population in the gut region. [10].

2.1 ***Counting of gut flagellates in a smear:***

The flagellate's population was counted using haemocytometer under Biological research microscope Series B-350 at magnifications 10 x 10 in four marked squares ($1mm^{2)}$ and found average in one square of the haemocytometer The following formulas was applied to calculate area and total number of flagellates in a smear.

Total number of flagellates = Average number of flagellates in 1W X Total area (πr^2) of the smear in mm^2.

3. RESULTS

3.1 Coptotermes heimi

The result of feeding of the termite, *C. heimi* on filter paper impregnated with resochin, entox, SDM, SQ and DV is given in Table 1. Tthe flagellates population in the workers after one day was 2542.4±118.3, 4585.4±267.8, 2587.8±103.5, 1929.5±101.5and 1566.3±81.21, respectively and it was significantly less as compared to zero hour of the experiment . After 48 and 72 hours of feeding on filter papers impregnated with these drugs the flagellate population decreased significantly and by 100% in all the cases except in entox where the population rate was 4222.2±103.5 and 4540±187.5. In control, the flagellates population decreased insignificantly and slightly and was 4568.14±83, 4505.49±119.5 and 208.58±88.5 after 24, 48 and 72 hours, respectively (Figure 1).The percentage mortality of *C. heimi* workers after 24 hours of feeding on the impregnated filter paper with resochin, entox, SDM, SQ and DV was 38.67±0.88, 100±0, 40.33±0.33, 31±0.58 and 19.66±0.72, respectively. After 48 hours the average mortality for the same drugs in the same sequences was 5±0.58, 100±0, 9.66±0.88, 13.66±0.72 and 16±0.58whereas after 72 hours of feeding, all the workers died in all the cases except in entox where the survival was 100±0.00 (figure 2).In the control, there was virtually no mortality of workers after 24 hours 48 hours and 72 hours of the experiment. The drugs fed workers were dissected and found no active live protozoans were present.

By feeding the soldiers of *C. heimi on* Resochin, Entox, SDM, SQ and DV, the data on the flagellate population shows that after 24 hours of the feeding on the impregnated filter papers, the flagellates population was 2315.±197, 2724±205, 2487.8±149, 2179.2±284 and 1906.8±156, respectively whereas after 48 hours of feeding the population was 2088.4±175, 2425.2±186, 2043±203, 2224.6±162 and 1725.2±150, respectively. After 72 hours of the experiment no flagellate was alive in the gut of soldiers. In the case of control group of soldiers almost the same mortality of their symbionts was observed as in the case of experimental groups after 24 hours and 48 hours i.e. 2315.4±196 and 1679.8±152 after 72 hours of the experiment all the flagellates were found dead (figure 3). On feeding

the soldiers of the *C. heimi* on the impregnated filter paper with resochin, entox, SDM, SQ and DV it was seen that the soldiers survived on these drugs for 24 hours but after 48 hours of feeding the average survival population of the soldiers was 40.66±0.67, 40.66±0.89, 47.66±0.89, 50±1.155 and 50±1.20 respectively and after 72 hours of the feeding all the soldiers died in all the cases. The control showed that the soldiers survived for 24 hours but after 48 hours 47±0.57 of them survived whereas after 72 hours of the experiment, all the soldiers in the control had also died (figure 4). Some of dead soldiers in both experimental as well as in control group, when dissected were found to contain a few a live protozoans.

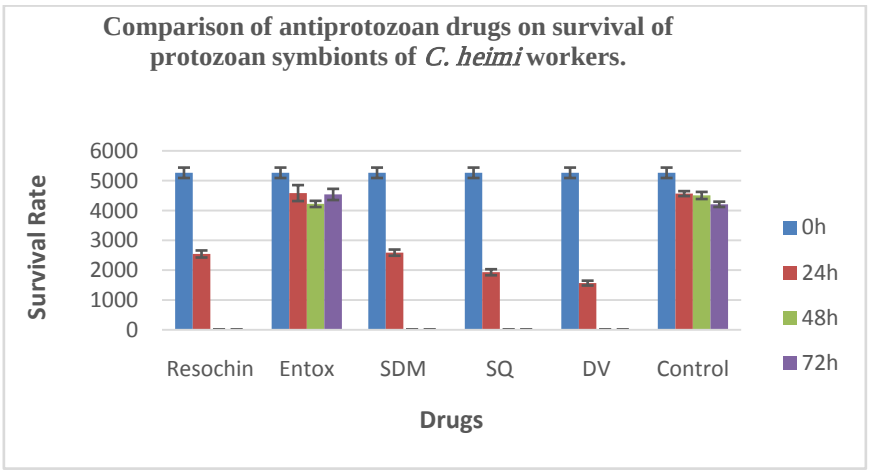

Figure 1. Effect of different antiprotozoan drugs on survival of protozoan symbionts of *C. heimi* workers.

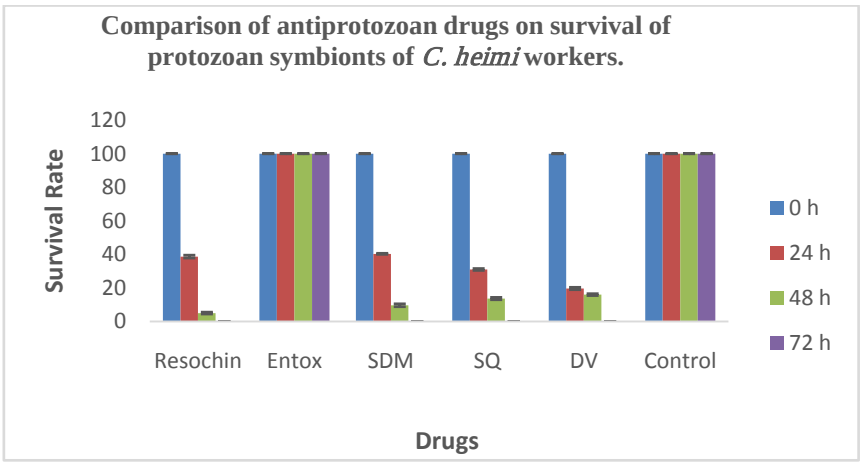

Figure 2. Effects of different antiprotozoan drugs on survival of *C. heimi* workers.

3.2 *Heterotermes indicola*

The anti-protozoan drugs Resochin, Entox, SDM, SQ and DV showed similar effects on workers and soldiers of *H. indicola* and also on the flagellates of these casts as were observed in the case of C. *heimi*. The flagellate population in *H. indicola* workers was decreased by 2292.7±180.4,

2996.4±231, 2837.5±101.5 and 3450.4±149.19 on feeding on Resochin, SDM, SQ and DV respectively 24 hours after feeding them on these drugs whereas after 48 hours the flagellates' population was decreased significantly and considerably by 100% in all the cases. The flagellate population in control decreased only slightly and insignificantly by 4767±160.51, 4731.6±358.87 and 4449.2±282.43 after 24, 48, and 72 hours respectively. The entox behaved similarly as in *C. heimi*, the average population flagellates in workers after 24, 48 and 72 hours was 5016.7±130, 4994±143 and 4721.6±174.65 respectively on entox impregnated filter paper (figure 5).

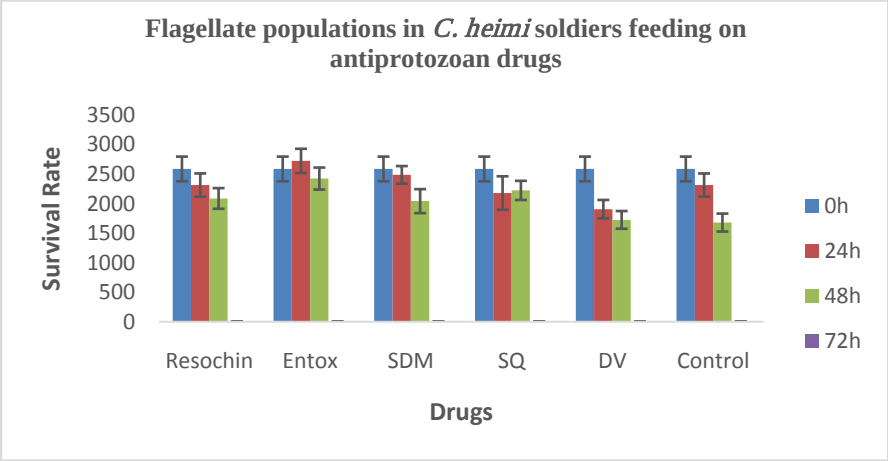

Figure 3. Flagellate populations in *C. heimi* soldiers feeding on antiprotozoan drugs.

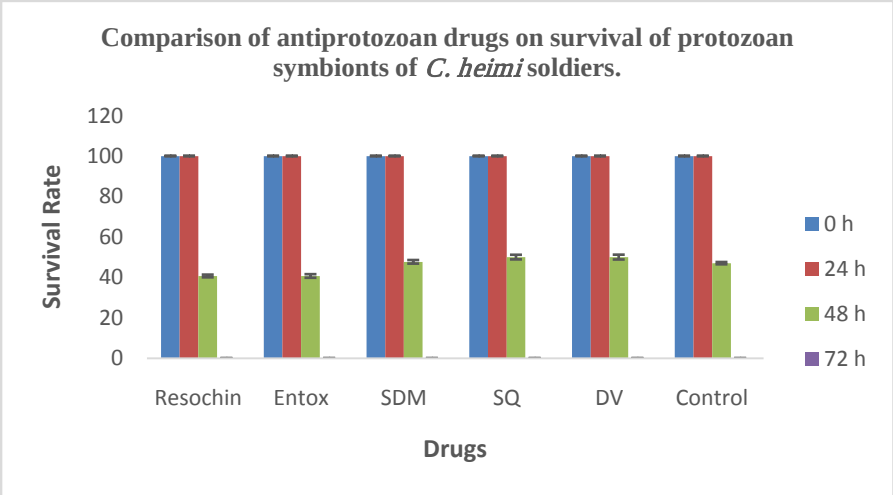

Figure 4. Effect of different antiprotozoan drugs on survival of *C. heimi* Soldiers.

The data on the survival of *Heterotermes indicola* workers showed that after 24 hours of feeding on the impregnated filter paper with resochin, SDM, SQ and DV was 37±1.20, 47±0.58, 46.66±0.88 and 35.33±0.34, respectively. After 48 hours the average was 18±1.15, 14.66±0.34, 9.66±0.88 and 17.66±0.88, respectively. Whereas after 72 hours of feeding on the drug impregnated filter paper, all the workers died in all the cases. In the case control there was virtually no mortality of workers after 48 hours and 72 hours of the experiment. The workers of *H. indicola* showed no mortality on feeding entox impregnated filter paper (figure 6). The flagellates' population of *Heterotermes indicola* soldiers

showed similar results as was observed in *C. heimi*. No significant mortality was seen after 24 and 48 hours but after 72 hours of the experiment all the soldiers died in all experimental groups and similarly in the control (figure 7).

The data on average mortality of the soldiers of *H. indicola* by feeding on the filter paper impregnated with Resochin, Entox, SDM, SQ and DV showed that the soldiers survived on these for 24 hours but after 48 hours of feeding the average mortality of the soldiers was 73±1.00, 77.66±0.88, 61.66±1.67, 74.66±1.45 and 66±0.58, respectively and after 72 hours of feeding all soldiers died in all the cases. The control also showed similar results to those of experimental. The soldiers survived for 24 hours but after 48 hours mortality were 67.33±1.20 and after 72 hours all of the soldiers died in the control as well as in experimental set (figure 8).

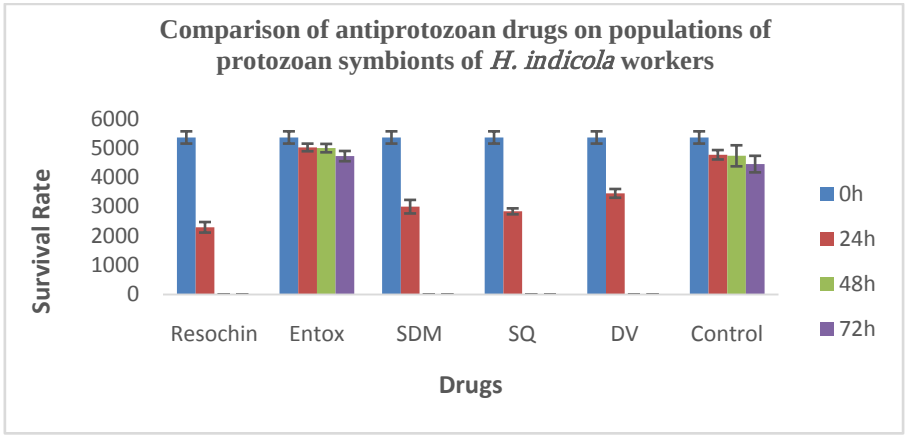

Figure 5. Effect of different antiprotozoan drugs on the population of protozoan symbionts of *H. indicola* workers.

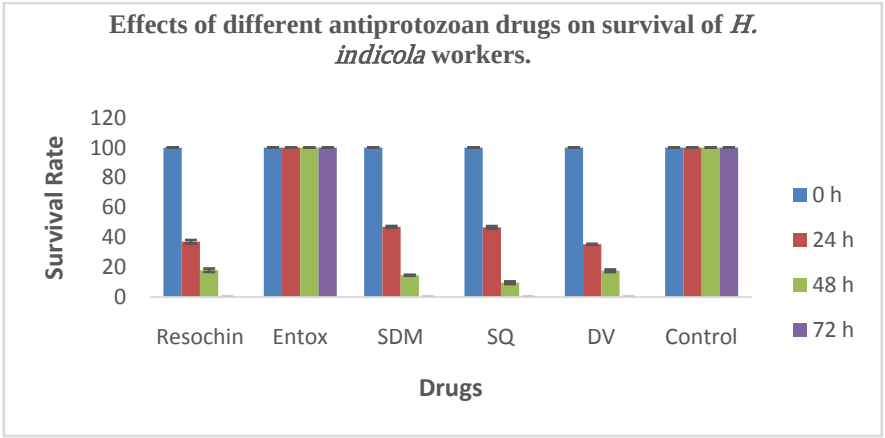

Figure 6. Effects of different antiprotozoan drugs on survival of *H. indicola* workers.

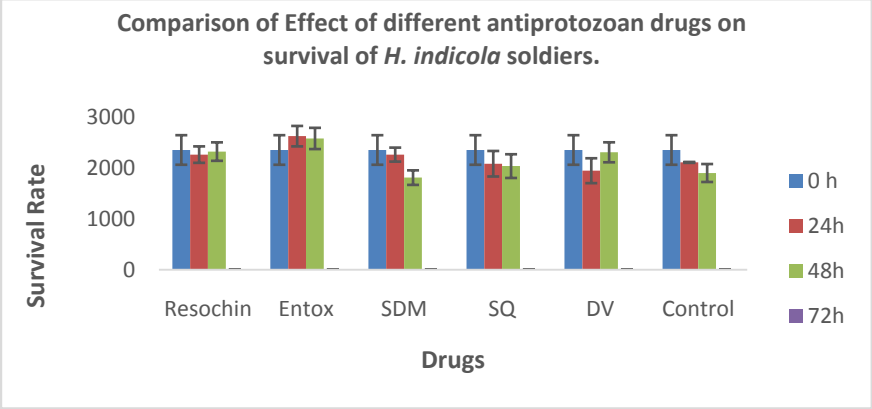

Figure 7. Effect of different antiprotozoan drugs on the population of protozoan symbionts of *H. indicola* soldiers.

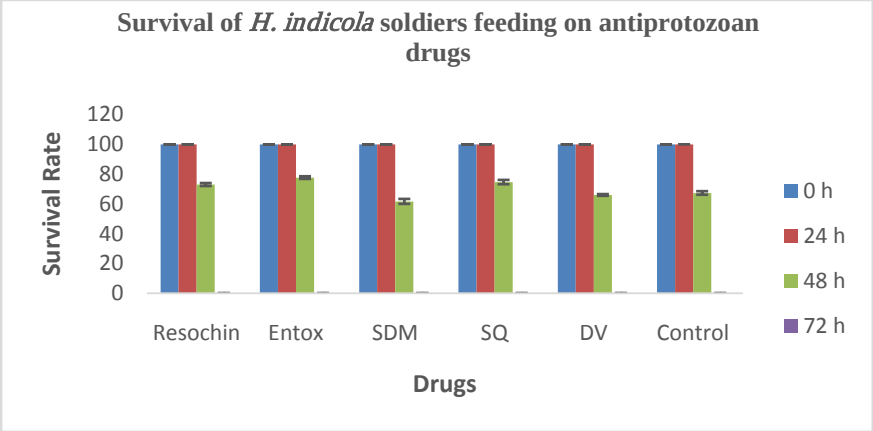

Figure 8. Effect of different antiprotozoan on the survival of *H. indicola* soldiers.

4. Discussion

The *C. heimi* and *H. indicola* harbor a great diversity of flagellates for the production of cellulases in their hind gut and are considered as lower termites. Their survival depends mainly on the survival of the gut flagellates. In the present investigations the forced feeding upon Resochin, entox, SDM, SQ and DV of workers of two termite species experimented, affected significantly the survival rates of their gut flagellates and their host termite workers also. It was examined that the increased mortality of termite workers in force-feeding on drugs impregnated filter paper appeared associated with the mortality of their gut flagellates. Thus, the termites underwent death because of the mortality of their flagellate protozoa. This appears to be true because termites and flagellates have developed a mutualistic type of association with each other and therefore defaunated worker termites do not survive for more than 2 days without flagellates [11].The research showed that Resochin, SDM, SQ and DV, diaveridine were all very effective and potent killers of the flagellates population of the termite species studied and their host termite workers. The protozoan symbionts were the first affected because after 48 hours of

exposure to the drugs, the population of flagellates was 100% dead, whereas the mortality of workers was 82 to 96% against various drugs used in this study for the same exposure period. The 100% mortality of workers occurred after 72 hr of exposure to these drugs. These observations clearly showed that these drugs i.e. Resochin, SDM, SQ and DV directly affected the flagellates and caused their death. The mortality of the workers appeared to be the result of secondary effect of death of the symbionts, which may have resulted in cutting the supply of nutrients to the workers. The data on the mortality of soldiers and their flagellate population indicates that the mortality was the result of starvation and not due to their exposure to drug impregnated filter paper as after 24 hrs no significant decrease in flagellates population P >0.05. The control with neat filter paper gave almost the same results of the mortality of soldiers and their flagellate population P>0.05. Since the soldier's mode of feeding is through workers, although the soldiers also have flagellates fauna in their gut but they get the same through anal feeding from workers and also their population is significantly less than the workers they get the soldiers did not feed on the impregnated filter paper or the immaculate (in control) at all. But after 48hrs of the experiment there was a significant decrease in population of soldiers and their flagellates. And their survival is not significant at both levels i.e. 0.05 and 0.01. It appeared that the soldiers and their fauna, in this case, died because of starvation. The result showed these drugs are effective anti-protozoan and anti-termite in the order: DV> SQ > SDM > Resochin. The variations in the efficacy were however, non-significant. The entox behaved differently from other anti-protozoan drugs. However there was no mortality of the termite workers which survived even after one week of feeding on entox impregnated filter paper and did not prove to be an effective anti-termite in these investigations probably because of its being insoluble in water

Many Organochlorine pesticides used in the past and are still in use in some countries. The United States has banned DDT, aldrin, dieldrin, arochlor, chlordane, heptachlor, mirex hexachlorobenzene, oxychlordane, toxaphene and others. The pesticides have very strong pungent odors, cause serious environmental hazards and are a source of many disorders too e.g. chlordane cause cancer, Monochrotophos, Chlorpyriphos, Dimethoate, and Endosulfan cause genotoxicity [12]. Polychlorinated Biphenyls and Organochlorine pesticides (e.g. α- and γ-chlordane, p, p′-DDT (dichlorodiphenyltrichloroethane), p, p′-DDE (dichlorodiphenyldichloroethylene), methoxychlor, and pentachlorophenol) remain persistent in indoor carpets and cause leukemia in children [13] These drugs also affect fetal reproductive and immune system. One popular termiticide chlordane causes cancer and banned in some countries. Therefore, such chemicals are not recommended for indoor applications [14].

The anti-protozoan drugs namely Resochin, Sulfaquinaxaline sodium, Sulfadimidine sodium and Diaveridine can be good substitutes for the organo phosphate chemical. The antimalarial drug e.g. resochin, quinine etc. can be quite acceptable for use in the residences and wooden households. These drugs can also be used for making the artificial wood as, heartwood, chipboard, wins board etc., to make them termite proof. The sulfonamides actually inhibit the formation of folic acid by blocking enzyme in microorganisms so causing their mortality whereas higher vertebrates takes folic acid orally with diet and is not synthesized in the body. However, if these sulfonamides enter into their body

directly or indirectly they will not cause a serious harm. According to the American Academy of Pediatrics, breastfeeding and sulfonamide use is compatible because sulfonamide excretion into human milk does not pose a significant risk to the healthy term neonate [15]. Resochin chemically is chloroquinine (CQ), N'-(7-chloroquinolin-4-yl)-N, N-diethyl-pentane-1, 4-diamine and was discovered in 1934 by Andersag and co-workers at the Bayer laboratories who named it "Resochin". It is used to cure malaria, rheumatoid arthritis, lupus erythematosus, chemotherapy, radiotherapy and is also being tried as an anti-cancerous [16-19[It causes auto digestion in microorganisms thus resulting in their death so it can serve as an acceptable replacement of termiticides. However before these drugs can be adopted for use in residence and for protecting wooden structure, a great deal of more research work on the efficacy and on stability of these drug is required to be carried out.

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