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LABORATORY AND FIELD EVALUATION OF HOUSEHOLD INSECTICIDE PRODUCTS AND PUBLIC HEALTH INSECTICIDES AGAINST VECTOR MOSQUITOES AND HOUSE FLIES (DIPTERA: CULICIDAE, MUSCIDAE)

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Abstract The effectiveness of insecticide formulations for public health pests is the key towards the success of vector control programmes. Evaluation of the efficacy of the abovementioned insecticide formulations by independent research institutes is very important before it can be registered and commercialized. Global and Regional trends on laboratory and field evaluation of household insecticide products and public health insecticides against vector mosquitoes and house flies are basically group into three phases. Phase I is a laboratory evaluation and should reflect the actual efficacy of the insecticide in the actual application. Glass Chamber method (0.7 x 0.7 x 0.7 m) and Peet-Grady Chamber method (1.8 x 1.8 x 1.8 cm) are among the most popular testing methods used for adult flying insects. Paper cup method and earthen jar method are used for the evaluation of larvicides. Phase II is only conducted after promising results are shown in the Phase I evaluation. Phase II studies consist of small scale field trial inclusive of indoor and outdoor assessments of space spray formulations, residual assessments of impregnated bed-nets, efficacy evaluation of personal protection products, earthen jar assessment of mosquito larvae and studies on toxic baits against housefly toxic. Village scale or larger scale field trials in Phase III are carried out only after the products are found to be effective at the Phase II assessment stage. Phase III studies are generally expected to provide the actual control outcome from the use of the pesticides such as reduction in the density of the target species or reduction in disease cases.
Key Words Pest control, vector control, fly control

INTRODUCTION

In developed countries such as North America, Europe, Japan, Australia, New Zealand and USSR/CIS, the urban population is expected to increase to more than 70% in year 2010 (Watson, 1993), thus reflecting the ever increasing importance of urban pests. Meanwhile, in developing countries such as Malaysia, the predominant pests are mosquito, followed by cockroaches, ants, rodents, flies and termites. Mosquitoes are the most important and abundant pest in urban, sub-urban and rural environment of Malaysia (Yap and Foo, 1984). With the increasing problems caused by urban pests, there is an important need for their control. Generally, urban pest control can be divided into five approaches, namely environmental management, source reduction, biological control, chemical control and integrated control.

Chemical control is still the main approach for urban pest control (Castle et al., 1999; Rozendaal, 1997; Marrs, 1993; Lee and Yap, 2003; Tidwell et al., 1994; WHO 1986, 1994). It can be divided into conventional measures which are generally conducted by the government; and personal protection measures which are practised by the community. Thermal fogging and ULV space spray are two of the most common methods conducted by the local health authority in an epidemic situation (Chavasse and Yap, 1997; Yap et al., 2000, 2003b). Other conventional measures include surface residual spray, larviciding inclusive of chemical and microbial agents, whilst household insecticide products (HIPs) and repellents are two major tools in personal protection measures (Yap et al., 1999a; Yap and Lee, 1999). In Malaysia, coils and aerosols are the two major household insecticide products that are readily used by consumers (Yap and Foo, 1984; Yap 1996, 1999; Yap et al., 2003a).

Biological control will give a long lasting effect if the biological agents can survive and recycle. It is environmentally friendly with minimal resistance problem. Many biological agents including predators, parasites and microbial agents have been assessed as bio-control agents for mosquitoes and other urban pests (Chapman, 1985; Weiser, 1991). However, the only bio-agents that are in operational use are bacteria *Bacillus thuringiensis* H-14 and *Bacillus sphaericus* 2362 (Cheong and Yap, 1985; Yap et al., 2001a; Glare and O'Callaghan 1998). In addition, specific microbial agents are targeted for certain pest species only (e.g. *Bacillus thuringiensis*

H-14 is for clean water-breeders such as *Aedes* and *Anopheles*, while *Bacillus sphaericus* 2362 is for polluted water-breeders such as *Culex* and *Mansonia* (Yap et al., 2003a, 1999b).

Insecticides have been heavily used as one of the most effective tools in vector control programmes (Halliday and Georghiou, 1985; Lawllen, 1960; Scott and Georghiou, 1986; Snell, 1999; Stevens and Stroud, 1967; Yap et al., 1983, 1994, 2001b; WHO, 1996). Although chemical control provides quick knockdown/mortality, resistant of mosquito against the use of insecticides have been widely reported (Omer et al., 1980; Poovaneswari and Lam, 1992; Raymond et al., 1987; WHO, 1964, 1980, 1992; Wirth et al., 1987). As such, more new insecticides/microbial agent have been developed. These need to be evaluated following a standard protocol before they can be commercialized. Presently, global and regional trends in the laboratory and field evaluation of household insecticide products and public health insecticides against vector mosquitoes and houseflies are basically grouped into three phases.

Phase I

Phase I which is a laboratory study should reflect the actual efficacy of the insecticide in its actual application. The glass chamber method (0.7 x 0.7 x 0.7 m) and the Peet-Grady Chamber method (1.8 x 1.8 x 1.8 cm) are among the most popular testing methods used in the Phase I evaluation for adult flying insects, while the paper cup method and earthen jar method are used for evaluating the efficacy of larvicides.

Glass Chamber. This test is conducted in a glass chamber measuring 70 x 70 x 70 cm. A total of 20 laboratory-cultured sucrose-fed adult female mosquitoes aged 2-5 days are released into the chamber. The insecticide is sprayed into the chamber by using a manual/electric atomizer. The discharge rate (gm/spray) of the sprayer is predetermined. Based on the dosage required, an estimated time of spray is discharged into the glass chamber. Knockdown of mosquitoes is observed at the indicated intervals up to 20 minutes. After 20 minutes, all mosquitoes are then collected and placed in cylindrical polyethylene containers with 10% sucrose pad. A further, single assessment of knockdown is made after 60 minutes. Mortality is observed after 24 h post-treatment. All tests to be conducted at a temperature of 26-28º C and relative humidity of 65-85%. A minimum of three tests is conducted. The knockdown values (KT50 and KT95) and regression slope are obtained using probit analysis (SPSS, 2000). Mean percentage of insect mortality value is subjected to arcsine transformation followed by comparison of means using the LSD test (SPSS, 2000).

Peet-Grady Chamber. This test is conducted in a Peet Grady Chamber measuring 180 x 180 x 180 cm. A total of 50 laboratory-cultured sucrose-fed adult female mosquitoes aged 2-5 days are released into the chamber. The insecticide is sprayed into the chamber by using a manual/electric atomizer. The discharge rate (gm/spray) of the sprayer is predetermined. Based on the dosage required, an estimated time of spray is discharged into the Peet-Grady Chamber through two introduction ports of the chamber. Knockdown of mosquitoes is observed at the indicated intervals up to 20 minutes. After 20 minutes, all mosquitoes are then collected and placed in cylindrical polyethylene containers with 10% sucrose pad. A further, single assessment of knockdown is made after 60 minutes. Mortality is observed after 24 hours post-treatment. All tests to be conducted at a temperature of 26-28º C and relative humidity of 65-85%. A minimum of three tests is conducted. The knockdown values (KT_{50} and KT_{95}) and regression slope are obtained using probit analysis (SPSS, 2000). Mean percentage of insect mortality value is subjected to arcsine transformation followed by comparison of means using the LSD test (SPSS, 2000).

Artificial Breeding Container. In this simulated laboratory study, various dosages of larvicides are used with untreated water as control. The study can be conducted using different sized containers. The water is allowed to stand for at least 48 hours prior to the experiment. Twenty laboratory cultured mosquito larvae (late third or early fourth instars) each of the mosquito species from well established laboratory colonies are introduced separately into both types of jars at specific times (24 h, day 3, day 7, week 2, week 3, week 4 until required residual periods). Larval mortality is recorded at 24 and 48 h post-treatment at each introduction period.

Two treatment regimes is adopted: 1 set of jars (3 per treatment dosage + 3 controls) which are not subjected to any replenishment of water (the water is only topped up to the water-level mark when evaporation occurs); the other set (also 3 jars per dosage + 3 controls) is given a daily replenishment of about 15% of water from the total tested volume so that there is a weekly turnover of the whole volume. The daily replenishment is to simulate daily usage of water in the container. Three sets of experiments are carried out with the residual activities being monitored for a required period. The trials at each dose tested are terminated when the mortality rate fall below 50%.

Phase II

Phase II evaluation is only conducted after promising results are shown in the Phase I evaluation. Phase II studies consist of small scale field trial which include indoor and outdoor assessment of space spray formulations, residual assessment of impregnated bed-nets, evaluation of the efficacy of personal protection products, earthen jar assessment of products against mosquito larvae and toxic bait studies against houseflies.

Outdoor Assessment of Space Spray Formulations. The test site for outdoor thermal fogging and ULV trials is an open space measuring more than 100 x 200 meters (2 hectares). The following environmental parameters are recorded for all the trials: time of spraying (1900 – 0000 h), temperature (25-30 ºC), relative humidity (70-90%) and rainfall (no rainfall when spraying). In addition, wind direction and velocity (0.5 – 3.0 m/s) is also recorded in the outdoor trial. For outdoor trials, either the thermal fogger or the cold fogger (ULV sprayer) is mounted on a vehicle with the sprayer head nozzle pointing upwards at an angle of 30 degrees to the horizontal plane. The vehicle travels at a distance of 200 meters perpendicular to the spray angle at a speed of 6-9 km/h.

Adults of *Aedes aegypti*, *Aedes albopictus* and *Culex quinquefasciatus* used in the study are from well established laboratory susceptible colonies. Sucrose-fed female mosquitoes aged 2-5 days are used for the adulticidal assessments. For larvicidal assessments, late third or early fourth instar larvae are used. The efficacy of the insecticide is assessed at 10, 25, 50, 75 and 100 meters (a total of 5 checkpoints) downwind of the spraying vehicle. Water sensitive papers are set at each check points as indicators of the presence of the insecticides at each of the check points.

The adulticidal effect is assessed by keeping twenty adult mosquitoes of the respective mosquito species in a cylindrical cage constructed of fine mesh fabric (nylon) with wire frame support (diameter 10 cm x height 15 cm x tapping cover 10 cm). Two mesh sizes are used. For *Aedes aegypti* and *Culex quinquefasciatus*, 1.2 mm mesh size is used, whereas for the *Aedes albopictus*, 0.5 mm mesh size is used. The respective caged mosquitoes are placed approximately 1.5 meters above the ground at each checkpoint. Knockdown of mosquitoes are read at 0, 10, 20 30, 40 and 60 minutes post-spraying. After field exposure of 30 minutes, the mosquitoes is brought back to the laboratory at a temperature of 26-28 ºC and relative humidity of 65-85% and transferred into clean polyethylene cups with 10% sucrose pad. The 0, 10, 20 and 30 minute knockdown readings are read in the field, while the 40 and 60 minute knockdown readings are read in the laboratory. The transportation time between field and laboratory is about 3 minutes. Mortality of mosquitoes is recorded at 24 h post treatment. The same protocol is followed for the control using water.

Larvicidal effect is assessed by placing twenty larvae of the respective mosquito species in separate paper cups on the ground below the adult cages. After field exposure of 30 minutes, the larvae are brought back to the laboratory and kept in a laboratory environment at a temperature of 26-28 ºC and relative humidity of 65-85%. Mortality of larvae is recorded at 24 h post treatment.

Teflon coated slides are placed approximately 1.5 meters above the ground at each check-point on a slide rotator for droplet deposition to determine droplet size. The slide rotators are switched on before spraying and switched off ten minutes post-spraying. The slides are kept in a slide holder box and brought back to the laboratory to assess the droplet size. The droplet size is read under a microscope and analyzed using a computer program created by Clarke Engineering Technologies in Microsoft Excel to determine the VMD (volume medium diameter) value of the droplet size in m. A minimum of 3 trials per formulation inclusive of control (water without insecticide) is conducted.

Indoor Thermal Fogging and ULV. The test site for indoor thermal fogging and ULV trials consists of residential houses. For indoor trials, portable thermal foggers and portable ULV cold fogger are used for the indoor thermal fogging trial and ULV trials, respectively. The trial is conducted by spraying indoors in the living room and kitchen. For each premise, an area of around 360 m^3 is sprayed. An appropriate discharge rate and spraying time are chosen to achieve the recommended dose of the formulations. A street with a row of houses is chosen for the spraying of each dosage of the insecticide. A similar street situated more than 50 m away from the treated street is chosen as the control lane.

Efficacy is assessed in five of a minimum of 20 houses chosen for spraying. The efficacy is assessed at two checkpoints for each assessed house, the first checkpoint is the living room and the second checkpoint is the kitchen of the premise, with the adult mosquitoes in cages and larvae in cups on the floor below the adult cages. Similar arrangement is prepared for the control lane. Adulticidal and larvicidal efficacy is assessed similar to the outdoor assessment. However, the knockdown of mosquitoes is only read at 0, 10, 20 and 30 minutes post-spraying. After field exposure (30 minutes), the mosquitoes are transferred into clean polyethylene cups with 10% sucrose pad in the field. A further, single assessment of knockdown is made after 60 minutes.

Mortality of the mosquitoes is recorded 24 hours post treatment. In the control lane, the above assessment was also carried out and recorded. Droplet size assessment was also carried out.

Mosquito Larvae Assessment. In this field simulated study, the larvicides are used with untreated water as a control. All of the earthen jars/plastic containers are set around the residential houses outdoors, under shade. The earthen jars/plastic containers are covered with a fine mesh net (mesh size less than 0.5 mm) to prevent field mosquitoes from breeding. The water is allowed to stand for at least 24 hours prior to the experiment. All formulations will first be weighted before being manually introduced onto the water surface in the jars/containers. Twenty five laboratory cultured mosquito larvae (third instar) of *Aedes aegypti* from well established laboratory colonies are introduced separately into both types of jars/containers at specific times (i.e. 24 hours, day 7, week 2, week 3, week 4 and subsequently biweekly up to a maximum period of 12 weeks). Larvae mortality is recorded at 24 and 48 hours post-treatment after each introduction period. Subsequently, adult emergence will also be observed and recorded until the next introduction of larvae. A day before the introduction of larvae at each period of time, water is topped up to the water-level mark for all treated/untreated jars/containers to compensate for evaporation. Five sets of experiments are carried out with residual activities being monitored for a maximum of 12 weeks. The trial for each formulation is terminated when mortality rate falls below 50%. For comparative efficacy, the percentage of mortality calculated is subjected to square root transformation followed by arcsine transformation and finally by comparison of means using the LSD test using the SPSS computer program.

Space-spray Machine Evaluation. The performance and effectiveness (bioefficacy and droplet size assessment) of the space sprayer is tested before operational use for a period of one year. The bioefficacy assessment protocol is the same as the outdoor assessment for space sprayer formulation. A thorough check of the machine essentially following the WHO guideline (WHO, 1990) is carried out to ensure that the machine is in good condition before the initiation of pre-use assessment. The performance and effectiveness (bioefficacy and droplet size) of the machine is assessed after six months of operational use. Intermediate-use pilot field trial is carried out at the USM football field based on the test protocol similar to pre-use assessment. The performance and effectiveness (bioefficacy and droplet size) of the machine is reassessed after one year of operational use by the Ministry of Health, the collaborative agency responsible for using the machine in actual spraying.

After the pre-use assessment, the machine is put through operational use for a period of one year. A set of tools necessary for the maintenance and repair of the machine with enough spare parts for routine replacement during normal use of the machine is supplied by the manufacturer. An adequate stock of insecticide formulation is used for operational control. In addition, the accessories for mixing the insecticide formulations such as buckets, funnels with strainers and jerrycans will also be supplied. Only recommended fuel (unleaded petrol) is used throughout the period of the operational use. Safety precautions for the operational use essentially follows WHO guidelines (WHO, 1990). The machine is mounted on a vehicle for the vector control operations. The application time, sprayed area, number of houses and population covered, locality, wind velocity, temperature, relative humidity, type of insecticides used, volume of insecticide sprayed and nozzle size is recorded for every operational use.

In between operational use, regular servicing and maintenance such as cleaning the machine, flushing of insecticide after use, periodical checks and preparation for storage is judiciously carried out and recorded. The servicing and maintenance is conducted by the company (local representative) according to the proposed schedule. Breakdowns, repairs and replacement of parts are also recorded.

Phase III

Village scale or larger scale field trials in Phase III should be carried out after pesticides are found to be effective at Phase II assessment. Phase III studies generally expected to provide the actual control outcome of the pesticide such as suppression of the density of the targeted vector species or reduction in disease cases.

Space Spray Efficacy Evaluated Using BLC Method. Before the application of space spray formulation in the targeted housing area, the mosquito population is assessed using the Bare Leg Catch (BLC) method. This method uses human baits that are required to expose both of their lower legs to allow mosquito to land. All landing mosquitoes are collected using test tube to assess the mosquito population. After confirmation of the existence of mosquito in sufficient numbers in the targeted residential area, the space spray application is initiated. Mosquito population is then assessed three, five and seven days post treatment, followed by a weekly assessment until percentage of reduction is below 50%. The effectiveness of the space spray can be assessed

based on the number of mosquitoes collected. In addition, the parous rate of the adult mosquitoes is also checked. If post-treatment parous rate parous rate is high, it implies that the control application is a failure. However, low parous rate indicates newly emerged mosquito.

Larviciding Trials. The trial is conducted in a residential area. This field trial is conducted in collaboration with the Ministry of Health and related community councils. A total of 100-150 houses are chosen for the mosquito breeding assessment. Ovitrap method (Yap, 1975) is used to determine the presence of dengue vectors (*Aedes aegypti* and *Aedes albopictus*) at the experimental sites. Existing household containers (e.g. water storage container) is checked for *Aedes* breeding. The number of mosquito larvae found in the existing household water containers is recorded before the introduction of larvicides at its optimum dose. All houses with positive mosquito breeding water containers (except for ten houses with the highest population to be used as untreated control houses) are treated with larvicides at the recommended dose. After treatment, the number of larvae found breeding in the respective treated existing water containers is recorded every 5 days up to a stipulated period of time. In the initial post-treatment, mortality is read at 24 and 72 hours in addition to the 5 days reading.

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