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学位論文名	発展途上国においてマラリアをはじめとする蚊媒介性疾患を制御することを目的とした簡単で低費用な蚊幼虫殺滅方法の研究
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論文内容の要旨

1 研究目的

Malaria and other mosquito-borne diseases such as dengue hemorrhagic fever (DHF) has been a major problem in undeveloped and developing countries. Many of malaria or DHF eradicating campaigns in the line of medical treatment, mosquito genetic modification and vaccination had not been successful. Vector control seemed to be a better solution in these countries. Many efforts of vector control had long been a great failure, since most of vector control efforts merely focus on DDT spraying or fogging when malaria or DHF outbreaks occurred. I was encouraged to find a more simple and low-cost alternative substances to be utilized in vector control. Copper had been widely use in daily life and have a safer toxicity range in vertebrae when compared with other insecticides. The capability of copper in low concentration to killed or injured mosquito larvae had encouraged me to use it in a safe and correct application to reduce mosquito outbreaks with minimal effect to human health and environment. This would be valuable addition to Integrative Vector Management (IVM).

2 研究方法

Anopheles stephensi Liston, 1901 (strain SDA 500) reared in our laboratory was used in majority of experiments in my studies. In my first and second studies of low concentration of copper larvicide capability towards mosquito larvae, guppy (Poecilia reticulata, Peters 1859) and medaka (Oryzias latipes, Temminck et Schlegel, 1846) fish was utilized to check copper ability to jeopardized mosquito larvae survival ability. Copper solution was prepared and diluted from standard 100 mM solutions and confirm the ppm level using Copper Measuring Device (Hanna Instruments, Tokyo, Japan) and Polarized Zeeman Flame Atomic Absorption Spectrophotometer (Hitachi Ltd, Tokyo, Japan). For my third studies of copper utilization for ovitraps, Aedes albopictus and Culex pipiens was also used and reared in our laboratory. Wild type Anopheles sinensis captured

in the field was utilized to confirm mosquito inability to detect and avoid copper. In my fourth studies of copper utilization to reduce Aedes albopictus number in Japanese cemeteries, copper was directly applied to flower vases in the cemeteries. The number of larvae prior and after the experiment was counted and statistically analyzed. All data in my studies was analyzed using student T-Test.

3 研究成果

Copper showed an adequate larvicide capability in a very low concentration (1.2 ppm). High mortality of larvae was achieved in both 1.2 ppm and 2.4 ppm concentrations. A lower concentration of less than 1 ppm (0.6 ppm) was not adequately kill the larva, but disrupted and jeopardized mosquito larvae capability to survive predation by larvivorous fish (guppy and medaka). Guppy and medaka can eat copper treated larvae significantly faster when compare to non-copper treated larvae. Copper also showed potency to be used in ovitraps. All three species of mosquitoes (An. stephensi, Ae. Albopictus, and Cx. Pipiens) showed >95% mortality in 96 hours after copper exposure (10 ppm) in the laboratory condition. Copper could not been detected nor avoided by wild mosquito. All the wild type Anopheles sinensis laid eggs randomly in the copper treated and non-copper treated water. Copper also reduced the number of Aedes albopictus larvae in Japanese cemeteries significantly. The copper treated flower vases in the cemeteries contain 50% less mosquito larvae when compared to the non-treated flower vases.

4 考察

Copper capability to kill mosquito larvae was achieved in a very low concentration starting from 1 ppm. This concentration is still below the toxic level to human (between 2-3 mg recommended daily allowance) and other vertebrae (sheep to rat) of 25 to 1000 ppm maximum tolerable levels. Below 0.6 ppm of concentration, copper no longer induced adequate mortality to mosquito larvae, but still disrupted and jeopardized their survival and diving ability. Loosing this ability made mosquito larvae more susceptible to predators, such as mosquito fish (guppy and medaka). It might be possible to combine this phenomenon, since fish are slightly more tolerant to copper in the concentration up to 0.26 ppm. Until this concentration, copper still had effect on mosquito larvae health, survival, and diving ability, but safe for small mosquito fish like medaka. It is also possible for fish to do acclimatization to copper, while mosquito larvae did not showed acclimatization capability. Utilizing copper in ovitraps is highly possible and promising, since copper showed adequate larvicide capability between 1 ppm to 10 ppm concentration. The possible mechanism of copper toxicity to mosquito larvae is copper binding to receptors in peritrophic matrix (pm) in the midgut of mosquito larvae, which then damage the midgut and disrupt their feeding process. No evidence

of detection or avoidance to copper by mosquito larvae. Wild anopheline mosquito laid eggs randomly in copper treated water vs control, which lead the possibility to utilized copper in ovitraps, to lure mosquito to breed there and kill the larvae in the process. Excessive copper consumption may pose health risk to human or high bioavailability water environment. Therefore, the Environment Protection Agency allowed copper to be applied in drinking water up to 1 ppm concentration limit. It should be considered to use copper only in limited place like ovitraps and low bioavailability water environment to prevent the health risk to human or the environment. Copper-based ovitraps or other copper utilization to kill mosquito larva should be carried out in responsible manner and careful control for Integrative Vector Management (IVM). Field tests and future researches are required to ensure the success and safety of this method. So far, copper capability to suppress mosquito larvae number in Japan cemeteries had shown promising field progress. Hopefully copper can be a simple, low-cost alternative to control mosquito outbreaks and reduce malaria, dengue and other mosquito-borne diseases in the future

5 結論

Copper might be a promising candidate for larvicide in the future. Many studies can be conducted regarding copper capability to kill mosquito larvae. It might be possible to combine copper-based ovitraps with mosquito attractants to increase success. Several study are currently focus on natural mosquito attractants, which would be a good pairing to copper-based ovitraps. The nature of copper as a readily available, daily use and low cost made it a suitable choice for undeveloped and developing countries. Responsible and comprehensive acts are needed to ensure the success in vector control and Integrative Vector Management.

論文審査の結果の要旨

本論文は、マラリア等の蚊媒介性疾患の流行地域において、媒介蚊を簡易かつ安価に駆除する方法の実用化を目指した、感染症制御上の重要性が高い研究内容に基づいている。研究目的は極めて明解であり、かつ世界的規模での影響力および社会的意義の大きい研究テーマであると言える。

申請者は、1) ハマダラカ幼虫の生存率に対する銅イオン濃度の影響を解明するとともに、水道水の水質基準値である 1ppm 以下の低濃度銅イオンで幼虫の弱体化が観察され、この弱体化した幼虫がグッピーによる捕食で短時間のうちに殺滅されることを見出した。2) メダカに対する致死的な影響が見られない 0.26ppm の低濃度銅イオン条件でもハマダラカ幼虫は弱体化し、メダカによる捕食で短時間のうちに殺滅させることに成功した。以上の結果は、低濃度銅イオンが蚊幼虫の駆除に有効であることを科学的に示しており、第 1 報および第 2 報として専門誌に報告済みである。しかし、魚類に対する安全域が狭いことも同時に示唆してお

り、環境中の多様な生物に対する影響が少なくないと推察される。そこで申請者は、ovitrap 内で 10ppm までの銅イオン溶液を用いることで環境への汚染を防ぎつつ ovitrap で産卵された蚊幼虫の駆除が可能であることの実証を試みた。その結果、3）供試蚊 3 種は銅イオン溶液入り ovitrap を忌避することなく産卵し、幼虫駆除効果が認められた。4）野外試験においても、銅イオン溶液入り ovitrap 内の蚊幼虫数は銅イオン未添加の対照 ovitrap よりも有意に低いことが証明された。

今後、実用化に向けては、さらに野外試験での調査対象として蚊成虫も含め、長期間の評価が必要ではあるが、本研究で得られた知見は、発展途上国でのマラリア等の媒介蚊の駆除に極めて有効な環境コントロール法の開発につながる可能性が高いと期待される。したがって、学術的価値にとどまらず、人類の健康と福祉に資する内容を包含するものとして、博士の学位を授与するに相応しいものと高く評価できる。

以上のことから、本申請論文は学位論文として合格であると判定された。

最終試験の結果の要旨

申請者の発表内容は論理的であり、目的とそれに対する実験計画と結果、および考察が明解に示された。実験背景の説明や結果解釈に必要な知識を十分に備えていることが窺われ、博士として独立して研究にあたるに相応しい素養を有していると判断された。審査員との間で、実験方法、データ解釈、統計解析等に関する質疑応答がなされた際には、いずれの質問に対しても適切な回答が述べられた。また、本研究の成果を実用化するまでの課題や将来的な構想についても、質疑応答の中で深い考察に基づく方向性が示された。

以上のことから、申請者は博士の学位に相応しい科学的知識と研究遂行能力、ならびに論理的思考と発表の能力を有することが明らかとなり、合格と判定された。