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Mini-review on the efficacy of aquatic macrophytes as mosquito larvicide

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Summary

Malaria is a mosquito-borne disease, which is endemic in Asia, Africa and Latin America. Vector control is the current strategy used for the eradication and elimination of malaria in these countries, but this control method has not proven to be effective, as malaria continues its increasing trend. Although chemical larvicide can also be used to eradicate the malaria vector at the larval stage, preventing the growth of mosquitoes into hematophagous adults, the continuous use of chemical insecticides leads to environmental pollution. It is therefore of paramount importance to identify effective, low-cost, biodegradable and environmentally friendly alternatives to chemical insecticides for the control of mosquito larvae.

This mini-review aims to assess the present and future of the use of macrophytes as a mosquito larvicide. We critically analyze the trend of malaria cases in sub-Saharan Africa and evaluate why botanical larvicides may contribute to the eradication of malaria in the region. The ecological role of macrophytes in the aquatic environment and their potential as botanical larvicide are explained in detail. The study illustrates that the macrophytes *Azolla pinnata, Pistia stratiotes, Eicchornia crassipes, Phragmites australis, Nelumbo nucifera, Nymphaea lotus, Typha latifolia* and *Leucas martinicensis* have been effectively used as larvicides against mosquito larvae. It is recommended that additional work be done to purify the biologically active components that are responsible for the larvicidal activity of these macrophytes, and future research should assess the potential of other macrophytes for effective utilization as larvicides.

Keywords: *Pistia stratiotes,* mosquito larvae, *Plasmodium falciparum*, aquatic ecosystem, *Anopheles*

Introduction

The invasive nature of macrophytes is problematic because they tend to dominate aquatic ecosystems to the detriment of the environment, economy and human health (Lamb et al., 2016; Ugya, 2015). Macrophytes left uncontrolled can cover the entire surface of water bodies, hindering water flow and sunlight penetration, which is detrimental to flora and fauna in the habitat. Different control methods have been used against these plants in the past, but recent research has focused on how to utilize the benefits associated with them (Chen et al., 2012; Hanks et al., 2015; Lareo, 1981). These macrophytes largely originate from malaria-endemic places and are regarded as invasive plants that impede economic and ecological improvement (Ugya et al., 2019b). A number of researches have shown how these macrophytes can be utilized for wastewater treatment while others have shown how these macrophytes can be used as a botanical larvicide against malaria (Ugya et al., 2015a; Ugya et al., 2016; Ma et al., 2019).

Malaria is a mosquito-borne disease which is endemic in Asia, Africa and Latin America. This disease has killed approximately 1.5-3 million people and infected 300-500 million (Scholte et al., 2003). Many affected countries have developed malaria control programs aimed at reducing the transmission of malaria to a level that is no longer a public health problem (Deletre et al., 2019).

Despite this effort, the progress made in malaria elimination and eradication has been minimal due to a lack of public health infrastructure and poor socioeconomic conditions in many affected countries. In addition, the malaria parasite has developed resistance to some anti-malaria drugs making it very difficult to develop an effective vaccine (Mwaiswelo et al., 2019; Silva et al., 2019).

Vector control is the current strategy used for the eradication and elimination of malaria. These control measures include the use of insecticide-treated nets and indoor residual spraying. These methods of malaria vector control have not proven to be effective, as malaria continues its increasing trend in endemic countries (Baia-da-Silva et al., 2019; Deletre et al., 2019; Mastrantonio et al., 2019).

The use of larvicides is critical in malaria vector control, killing mosquitoes in the juvenile stage and preventing their growth into hematophagous adults. Larvicides provide an effective control measure because at the larval stage mosquitoes are bound to aquatic habitat, making control strategies and application easier and more efficient (Unlu et al., 2019). However, heavy use of chemical insecticides in the control of mosquito larvae has led to environmental pollution which is detrimental to human health. It is therefore paramount to identify effective, low-cost, biodegradable and environmentally friendly alternatives to chemical insecticide for the control of mosquito larvae (Pandiyan et al., 2019; Pavela et al., 2019; Weeks et al., 2019).

Numerous studies have demonstrated the efficiency of different plants used as larvicides, repellants and ovideterrents with minimal environmental impact (Hari and Mathew, 2018). Macrophytes can be utilized for the production of botanical insecticide, such as larvicides, repellants or ovideterrents, since the plants are abundant due to their fast growing rate (Turnipseed et al., 2018). This mini-review aims to assess the present and future use of macrophytes as larvicide against mosquitoes.

Malaria in Sub-Saharan Africa and the importance of botanical larvicide

Malaria is a deadly mosquito-borne disease that is caused by a parasite belonging to the genus *Plasmodium*. There are five known species of *Plasmodium* that cause malaria in humans, namely *falciparum*, *vivax*, *ovale*, *malariae* and *knowlesi* (Walker et al., 2014). The disease is endemic in 87 countries and in 2017 ledto the death of 435.000 people, out of which 404.550 cases are reported to have come from sub-Saharan Africa (WHO, 2018). WHO and the governments of endemic countries have spent an estimated 4 billion USD for malaria control and eradication, but it is unlikely that the goals of the WHO Global Technical Strategy for Malaria 2016-2030 will be achieved based on the trend of death cases shown in Fig 1. Despite the large sum invested in the program, Tanzania has recorded no

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decrease in deaths resulting from malaria, while Angola, Benin and Kenya recorded an increase in death cases from 2010 to 2017. The death cases reduction trend is insignificant in countries like Cameroon, Congo, DR Congo, Mali, Niger, Mozambique and Sierra Leone (Fig. 1). Countries such as Burkina Faso, Uganda, Central Africa Republic, Chad, Coted'Ivoir, Ethiopia, Equatorial Guinea, Ghana and Nigeria report a significant reduction in death cases initially, from 2010 to 2012, but from 2014 to 2017, the progress was minimal.

Vector control has been shown to be the most effective strategy for reducing malaria transmission (PIMENTA et al., 2015). The two forms of vector control employed in most sub-Saharan countries are the use of insecticide-treated mosquito nets and indoor spraying of insecticide. These vector control methods may be effective in the reduction of malaria, but complete eradication will be virtually impossible because the malaria vector is developing resistance genes to insecticide, and the continuous application of insecticides tends to have adverse environmental effects (FULLMAN et al., 2013; N'GUESSAN et al., 2007).

The use of larvicides to eradicate mosquito at juvenile stage is significant because mosquito at this stage are confined to particular places of the environment. Several synthetic chemical agents have been shown to be effective in killing of mosquito larval but the adverse effect associated with the fate of the pollutants associated with the use of synthetic chemical agent is worrisome. Recent studies have focus on how to replace the use of synthetic chemical agents with a more ecological friendly method of controlling mosquito at the larval stage (PANDIYAN et al., 2019; PAVELA et al., 2019; WEEKS et al., 2019). The use of plant material as an alternative larvicide to synthetic chemical agent is promising, effective, easily accessible and environmentally friendly. Past research (PAVELA, 2015) has summarized findings regarding the potential of plant phytochemicals, including steroids, alkaloids, terpenes and phenolic constituents, for use as larvicides. The action of botanical insecticides depends on the plant species, parts extracted, collection site and solvent used for extraction (BENELLI et al., 2015; STEVENSON et al., 2017).

Ecological role of macrophytes in the aquatic environment

Plants that are able to survive in or around water bodies are referred to as aquatic macrophytes (OSTI et al., 2018). Macrophytes are classified into four major groups, namely emergent (e.g., *Typha angustifolia*), floating-leaved (e.g., *Nymphaea lotus*), submerged (e.g., *Ceratophyllum submersum*) and free-floating (e.g., *Pistia stratiotes*) (PULZATTO et al., 2018; UGYA et al., 2015b). These plants play a vital role in aquatic ecosystems due to their ability to utilize dissolved nutrients such as nitrogen and phosphorus for metabolic activities (HILL, 1979; UGYA et al., 2019a). Utilizing dissolved nutrients prevents algal blooms and the subsequent death of fish and aquatic mammals that may result due to the toxicity of microcystin produced by toxic algae (CHIA et al., 2019; TURNER et al., 2018).

Macrophytes also indirectly aid the efficiency of nutrient cycling in aquatic environments. They are able to retain solids in their roots and shoots, and their presence enhances suspended solid sedimentation by protecting against wind and waves, thereby reducing current velocity and the rate of erosion (THOMAZ and CUNHA, 2010; ZHU et al., 2015). Macrophytes indirectly enhance the cycling of nitrogen due to the presence of denitrifying bacteria inhabiting their roots and shoots. These denitrifying bacteria aid in the conversion of nitrate in the water into atmospheric nitrogen, thereby depleting the fertility of the water and limiting the growth of toxic algae (HALLIN et al., 2015). (HORPPILA et al., 2013) demonstrate that macrophytes (*Phragmites australis*) are associated with spatial heterogeneity in aquatic ecosystems. The uneven distribution of species within an aquatic ecosystem resulting from the presence of macrophytes increases the richness of aquatic organisms because spatial heterogeneity enables species to coexist with minimal competition (WANG et al., 2016). Macrophytes also provide shelter, breeding sites and sources of food for aquatic macro organisms (O'HARE et al., 2018).

The potential and efficacy of macrophytes as botanical larvicide

The use of macrophytes as a tool for the control of mosquito larvae in developing countries is promising. As summarized in Tab. 1 and

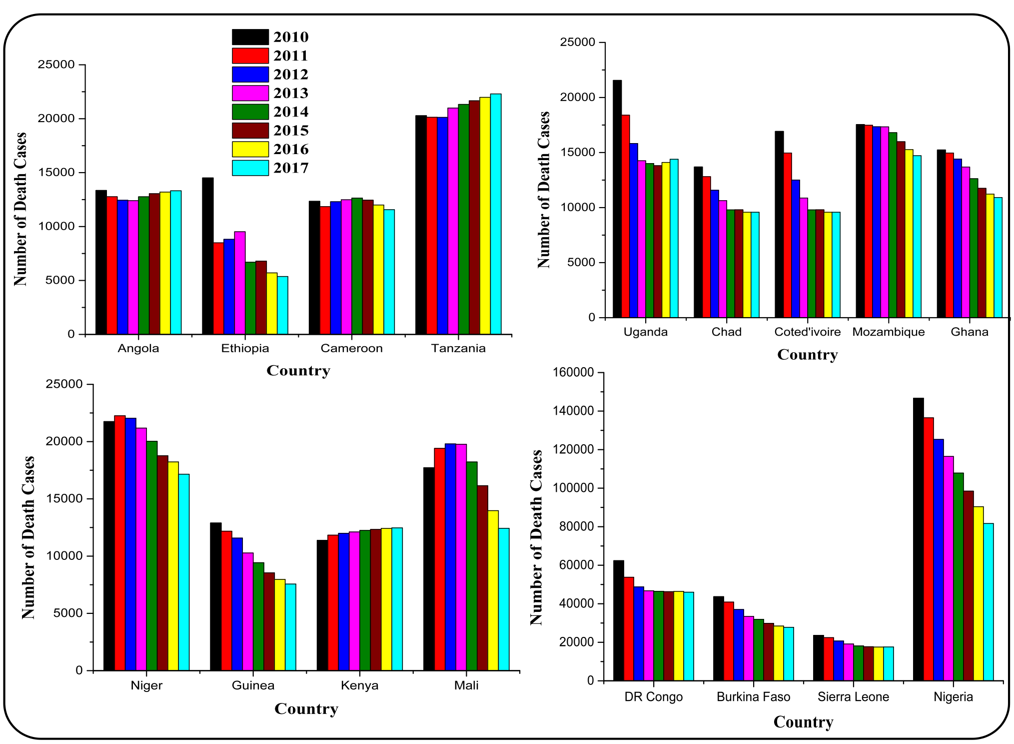

Fig. 1: Trend of Malaria Disease in sub-Saharan Africa

Tab. 1: Macrophytes with larvidal potential

SN	Name of macrophyte	Mosquito larvae	References
1	*Azolla pinnata*	*Aedes aegypti*	(HUSNA ZULKRNIN et al., 2018)
2	*Pistia stratiotes*	*Anopheles spp*.	(MA et al., 2019)
3	*Eicchornia crassipes*	*Culex quinquefasciatus Say*	(JAYANTHI et al., 2012)
4	*Phragmites australis*	*Culex pipiens*	(BREAM et al., 2009)
5	*Nelumbo nucifera*	*Anopheles stephensi*	(ANUSHREE et al., 2014)
6	*Nymphaea lotus*	*Anopheles spp*.	(YAKUBU et al., 2017)
7	*Typha latifolia*	*Anopheles spp*.	(IMAM and TAJUDDEEN, 2013)
8	*Leucas martinicensis*	*Anopheles spp*.	(IMAM and TAJUDDEEN, 2013)

Tab. 2: Characterization of phytochemicals present in different leaves extract of macrophytes

SN	Macrophytes	Acetone	Ethanol	Methanol	N-Butyl	References
1	*Eicchornia crassipes*	alkaloid, tannin and terpenoids	phenol, terpenoids, sterols	flavonoids, phenol, tannins, terpenoids	flavonoids, phenol, tannins, terpenoids	(HAGGAG et al., 2017; TULIKA and MALA, 2017a)
2	*Pistia stratiotes*	alkaloids, flavonoids, glycosides, photobatannins	steroids, alkaloids, terpenoids, flavonoids	tannins, sterols, terpenoids, alkaloids, flavonoids	-	(MA et al., 2019; TULIKA and MALA, 2017b)
3	*Potamogeton Specie*	-	phenol, flavonoids, tannins, terpenoids, alkaloids	phenol, flavonoids, tannins	-	(PAUL et al., 2015; QAIS et al., 1998)
4	*Nymphaea species*	phenol, flavonoids, tannins, saponins, alkaloids, steroids	flavonoids, tannins, saponins, tarpenoids, photobatannins, resin	phenol, flavonoids, tannins, saponins, alkaloids, steroids	phenols, flavonoids, tannins, glycosides, saponins, alkaloids, steroids	(AFOLAYAN et al., 2013; PARIMALA and SHOBA, 2013; YAKUBU et al., 2017)
5	*Typha latifolia*	tannins, steroids, saponins, alkaloids, glycosides	photobatannins, resin, alkaloids, steroids, tannins	alkaloids, tannins, steroids, phenols, saponins, flavonoids		(IMAM and TAJUDDEEN, 2013; RAMESH et al., 2013; WANGILA, 2017)
6	*Leucas martinicensis*		flavonoids, tannins, steroids, photobatannins, resin			(IMAM and TAJUDDEEN, 2013)
7	*Azolla pinnata*	phenol, steroids	phenols, flavonoids, saponins, steroids	phenol, flavonoids, tannin, saponins	-	(THIRIPURASUNDARI and PADMINI, 2018)

Tab. 3, a number of studies have demonstrated the efficacy of macrophytes as mosquito larvicide. The potential of macrophytes has been linked to the presence of phytochemicals (MA et al., 2019), which are bioactive compounds present in plant parts that have long been shown to possess insecticide potential (GHOSH et al., 2012). Phytochemicals extracted from different plants species have long been used in mosquito control (ISMAN, 1997). However, the discovery of DDT in 1939 overshadowed the use of phytochemical extracts for mosquito control (BERENBAUM, 1985).

Macrophytes produce numerous phytochemicals that possess medicinal and pesticidal properties. A large number of macrophytes have been shown to produce phytochemicals, such as flavonoids, tannins, saponins, alkaloids, resin, glycosides, phenol and phlobatannin, that cause high mortality when used as larvicides against mosquito larvae (MA et al., 2019). Flavonoids, also referred to as bioflavonoids, are secondary metabolites that have been shown by researchers such as (HUANG et al., 2015) to be present in macrophytes. They are produced by the phenylpropanoid metabolic pathway whereby phenylalanine is converted into 4-coumaroyl-CoA, which combines with malonyl CoA to produce chalcones (a backbone of flavonoids containing two rings). The pathway is then subjected to series of enzymatic actions by anthocyanidin reductase, chalcone isomerase, dihydrokaempferol-4-reductase, flavones synthase and others, leading to the production of products such as flavonones, dihydroflavonol, anthocyanins, flavan-3-ols, and proanthocyanidins (GUTHA et al., 2010; VERVERIDIS et al., 2007).

Anthocyanins, flavonones and dihydroflavonols are the major members of the flavonoids family, and they play a crucial role in the larvicidal potential of macrophytes (BATRA and SHARMA, 2013). Anthocyanins have been shown (KONG et al., 2003) to be produce in response to biotic and abiotic stresses. The strong antioxidant potential of macrophytes' anthocyanin is due to the presence of positively charged oxygen atoms (SADILOVA et al., 2006). Flavonones are produced as a result of the action of chalcone isomerase on chalcone-like compounds (FALCONE FERREYRA et al., 2012; PETRUSSA et al., 2013), while dihydroflavonols are produced as a result of the action of flavonone-3-dioxygenase, flavonol synthase and dihydroflavonol-4-reductase (TIAN et al., 2015). The high mortality of mosquito larvae resulting from exposure to extracts of macrophyte leaves recorded by most research is attributed to the larvae feeding on high concentrations of anthocyanin, flavonones and dihydroflavonols (WANG et al., 2013).

Tannins are another phytochemical that have been found in high concentration in macrophytes (YAKUBU et al., 2017). They are produced in the tannose, which is a chloroplast-derived organelle (BRILLOUET et al., 2013; HILLIS, 1958) located in the vacuole or surface wax of plants (BRILLOUET et al., 2013). These phytochemicals have a high level of astringency, which could be the reason most plant extracts containing high levels of tannin are highly effective as larvicides

Tab. 3: Larvicidal efficacies of aquatic macrophytes leaves extracts

SN	Macrophytes	Extract	LC50 (ppm)	References
1	*Pistia stratiotes*	ethanol	63.3	(Imam and Tajuddeen, 2013)
2	*Typha latifolia*	ethanol	68.1	(Imam and Tajuddeen, 2013)
3	*Pistia stratiotes*	ethyl acetate	14.80	(Ma et al., 2019)
4	*Azolla pinnata*	acetone	1072	(Ravi et al., 2018)
5	*Eicchornia crassipes*	petroleum ether	71.43	(Jayanthi et al., 2012)
6	*Azolla pinnata*	-	1267	(Husna Zulkrnin et al., 2018)
7	*Phragmites australis*	petroleum ether	60.06	(Bream et al., 2009)
8	*Phragmites australis*	ethanol	98.72	(Bream et al., 2009)
9	*Nymphaea lotus*	ethanol	62.8	(Yakubu et al., 2017)
10	*Azolla pinnata*	methanol	867	(Ravi et al., 2018)
11	*Eicchornia crassipes*	ethyl acetate	94.68	(Jayanthi et al., 2012)
12	*Eicchornia crassipes*	ethanol	152.15	(Jayanthi et al., 2012)
13	*Eicchornia crassipes*	methanol	173.35	(Jayanthi et al., 2012)

(Soares et al., 2012). The efficacy of phytochemicals extracted from macrophytes against mosquito larvae is dependent on the part of macrophyte used, the growth stage of the macrophyte and the solvents used during the preparation of the macrophyte extract, as shown in Tab. 2 (Daboor and Haroon, 2012; Haroon and Abdel-Aal, 2016). Saponin is a chemical compound that has been identified by (Yakubu et al., 2017) as present in macrophytes. The role of this phytochemical is to protect the macrophyte against predators and parasites due to it bitter taste and amphipathic nature. The amphipathic nature of saponin also contributes to the larvicidal potential of macrophyte extracts (Lorent et al., 2014).

Alkaloids are another bioactive compound found in the extract of macrophytes, as shown by (Ma et al., 2019). These bioactive compounds contain mostly nitrogen, although other elements such as oxygen, sulphur, chlorine, bromine and phosphorus may sometimes be present in the crude extract of alkaloids (Franck, 1967). The extract of alkaloid is an important pharmacological tool used for its antimalarial, anticancer and antibacterial properties (Cushnie et al., 2014). Alkaloid salts such as nicotine and anabasine have been widely used as pesticides, although the high human toxicity associated with these salts discourages their use (Duke et al., 2010). The pharmacological activities associated with alkaloids result from alkaloids' tendency to attack pathogens and prevent them from infecting human hosts. This high toxicity to pathogens and insects could explain why most macrophyte extracts with high or low concentration of alkaloids tend to be associated with high mosquito larvae mortality.

Resin is another bioactive compound shown by many researchers to be present in extracts of aquatic macrophytes. Resin also contributes to the larvicidal potential of extracts due to the presence of terpenes and resin acid, which protect plants against pathogens and insects (Klemens and Dieter, 2000). The protective role of resin is also part of the reason why macrophyte extracts are highly effective as mosquito larvicide.

Conclusion

Many control and prevention measures have been employed to manage the nuisance caused by mosquitoes. Inorganic pest control has remained the most effective tool, although it tends to have adverse side effects on humans and other living organisms in the environment. This mini-review demonstrates that extracts of macrophytes are active against mosquito larvae, and hence, these macrophytes could be an effective mosquito larvicide as they have also been found harmless to other aquatic organisms. Additional work should be done to further purify the biologically active components that are responsible for the larvicidal activity of these macrophytes, and more research should be done with respect to the potential of other macrophytes for effective utilization and eradication of the malaria vector.

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