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Deviation in the Metabolite Activity and Decreases in *Escherichia coli* Load in *Anopheles* Mosquitoes After Feeding with Blood Meal Treated with Nanocurcumin

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Abstract | The current study was performed to identify changes in metabolic activities and to measure the load of pathogens of *E. coli* as an example, in *Anopheles* mosquitoes after feeding with nanocurcumin-treated blood meal (NBM). The study included the use of 216 female mosquitoes divided randomly into six replicates for each group. The groups were a 6hr-ealier provision of NBM before *E. coli* exposure, the 6E group, in which NBM was provided to mosquitoes 6hrs before bacterial exposure. There was a group in which both *E. coli* exposure (ECE) and NBM were provided at the same time, the 0T group. In addition, there was a 6hr-laterNBM provision after ECE, the 6L group. Moreover, there was a 24hr-laterNBM provision after ECE in the 24L group. Finally, there were control groups, in which one group was supplied with sterile water only, the SW group, and a group that received no-NBM but ECE only, the nNBM group. For all treatments, NBM or ECE was only continued for 30 min. After the end of the experiment (24hrs after the supply of the NBM), the midgut of each mosquito was dissected. A test of the *E. coli* colony-forming units (CFUs) was performed for all groups. The metabolites were detected using mass spectrometry (MS). The results revealed significant (p<0.05) increases in the *E. coli* CFUs of the NNBM group when compared with those from the SW group. These levels decreased significantly (p<0.05) in all groups, especially in the 0T group. In the case of the metabolic activity, significant (p<0.05) increases were seen in the abundance of pantothenic acid, proline, and trehalose in the groups treated with NBM. The current study provides important findings that nanocurcumin shows protective and treatment activities against *E. coli* load and that comes with increases in the abundance of certain important metabolites in the midgut of mosquitoes.

Keywords | *Anopheles*, Mosquito-borne infections, Herbal insecticides

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INTRODUCTION

The presence of insect species presents several challenges to human and animal well-being, including potential threats to human health, food security, the preservation of threatened species, economic ramifications, and the sustainability of environments (Zhang *et al*., 2022). These species are sometimes referred to as exotic or imported organisms. Whether intentionally, accidentally, or via commerce, the human species is to blame for their emergence

(Venette and Hutchison, 2021). In addition to the evident threat they pose to biodiversity, the introduction of these insects also presents a substantial threat to the biosecurity of a country (Siddiqui *et al*., 2021). The fast growth of global commerce and human networking has facilitated the unprecedented transportation of species to previously untouched places around the planet. The recognition of the detrimental impacts of introduced species on indigenous biodiversity and the subsequent revenue losses in several countries has seen an upward trajectory in the last twenty years (Rao *et al*., 2018). Recent studies on the evolutionary genetics of invading species have shown that the capacity to adapt to natural selection could have more significance in facilitating the effective colonization of some imported species compared to general physiological flexibility or tolerance (Pimental *et al*., 2005; Marbuah *et al*., 2014). Consequently, the accidental consequence of this phenomenon is the enhancement of the invasive species' capacity for evolution, facilitating its rapid proliferation and geographic expansion within the introduced region (Lavergne and Molofsky, 2007; Hafez *et al*., 2022).

The persistence of illnesses transmitted by mosquitoes continues to pose a significant challenge to global public health since the development of effective vaccinations and treatments for many diseases remains limited (Reid and Mckenzie, 2016; Jawad and Alfatlawi, 2023). However, it is important to note that these illnesses may be prevented by measures such as the avoidance of mosquito bites and the elimination of possible vectors using pesticides (Richards *et al*., 2017; Dusfour *et al*., 2019). These strategies are considered essential components of integrated mosquito management (IMM) and have a significant role in reducing the incidence of these diseases. Nevertheless, the occurrence of control failures may be attributed to variables such as insecticide resistance (IR), inadequate pesticide application processes, and several other factors. It is worth noting that the underreporting of these failures might be partially attributed to resource constraints (Corbel *et al*., 2016; Jasim *et al*., 2023). Regular and efficient monitoring of mosquitoes for IR is crucial in order to gather information that may guide choices on mosquito management (Corbel *et al*., 2019; Jawad and Alfatlawi, 2023).

Mosquitoes encounter insecticides from different sources, including government-operated vector control programs, private pest control services for households, and commercial establishments, as well as approaches in agricultural settings (Depres *et al*., 2007; Dunbar *et al*., 2018).

Environmental researchers and agriculturalists possess knowledge of the enduring consequences of pesticides and the ramifications of the release of toxic chemicals into the environment. The rising prevalence of cancers and other chronic illnesses during the last half-century or so may be linked not only to increasing numbers of people but also to the use of pesticidal substances, which may enhance the effects of carcinogens (Jordahl *et al*., 1997; Li *et al*., 2008; Yadav *et al*., 2015). Wetland polluted with pesticidal waste poses a significant risk to both the aquatic ecology and drinking water supplies. Water pollution is a phenomenon characterized by the detrimental impact on water bodies resulting from the introduction of substantial amounts of foreign substances into waterways. Several remediation techniques, including leaching and landfilling, have been used to mitigate the adverse effects caused by the deposition of hazardous chemical contaminants in soil. Nevertheless, the implementation of soil remediation techniques including the elimination of harmful substances and the treatment of debris is both labour-intensive and financially burdensome (Gianfreda and Rao, 2004; Jie and Conrad, 2009; Jacobsen and Hjeluso, 2014; Cycon *et al*., 2019; Jayaraj *et al*., 2019).

The present scholarly investigations in the field of mosquito control are now fouced on comprehending the resistance of mosquitoes to synthetic pesticides and inventing innovative approaches to address these resistance concerns. The scientific community is increasingly focused on natural chemicals that exhibit superior efficacy and lower toxicity compared to their synthetic counterparts. The application of bioinsecticides, which consist of botanical or plant-derived substances, has emerged as a viable option owing to their limited adverse impacts on human well-being and the surrounding environment (Adeleke *et al*., 2008; Ayilara *et al*., 2014; Laxmishree and Nandita, 2017).

The current study was performed to identify changes in the metabolic activities and to measure the load of pathogens, *E. coli* as an example, in *Anopheles* mosquitoes after feeding with nanocurcumin-treated blood meal (NBM). Some important applications of nanocurcumin are wound healing, anti-inflammatory effects, drug delivery, antioxidant effects, etc. (Laxmishree and Nandita, 2017). Synthesis methods of nano-curcumin can be categorized as traditional methods and new and upcoming methods. In the traditional methods of nano-curcumin, the methods are: the ultrasonication method, sonicated nano-emulsion, the co-precipitation method, solvent-evaporation, solvent diffusion, the nanoprecipitation method, the simple solvent diffusion method, the micro-emulsification solvent evaporation method, the emulsion-diffusion method, the rotating pin micro-emulsification method, and the supercritical fluid technologies. In 2009, the first curcumin nanoparticles were produced by coating the curcumin complex with polymer-encasing nanoparticles. Very few methods for nano-curcumin have been reported in the literature.

Curcumin can also be dissolved in mono-, di-, and triglycerides, and then simply be atomized to yield nanoemulsions of curcumin. This method yields the nanoemulsion, which is a thermodynamically controlled stable carrier system with high bioactivity. In the nanoemulsion process, tricurin is not dissolved but exposed to an internal surface, which is very large and responsible for the water solubility of curcumin. This method is in principle quite simple and only a mixer is required. To further scale up the production of tricurin, an electrostatic atomizer is required, which is commercially available and has a capacity of several liters per hour.

MATERIALS AND METHODS

Experiment

The study included the use of 216 female mosquitoes (a few numbers were reared until reached a number higher than 216) divided randomly into 6 replicates for each group. Nanocurcumin was used from an industry product bought locally. The groups were a 6hr-ealier provision of NBM before *E. coli* exposure, 6E group, in which NBM was provided to mosquitoes 6hrs before bacterial exposure. There was a group, in which both ECE and NBM were provided at the same time, 0T group. In addition, there was a 6hr-later-NBM provision after ECE, 6L group. Moreover, there was a 24hr-later-NBM provision after ECE, 24L group. Finally, there were control groups, in which one group was supplied with sterile water only, SW group, and a group that received no-NBM but ECE only, NNBM group. For all treatments, NBM (8µg/ml) (Negahdari *et al*., 2020) or ECE (1%) (Souvannaseng *et al*., 2018) was only continued for 30 min. After the end of the experiment (24 h after the supply of the NBM), the midgut for each mosquito was dissected.

E. coli colony forming unit and mass spectrometry

A test of the *E. coli* CFUs was performed for all groups according to Othman *et al*. (2023). The metabolites were detected using MS based on methods by Souvannaseng *et al*. (2018).

Statistical analysis

The study data were analyzed and graphed using GraphPad Prism (v9). The Mean±SEM was used to present data. The level of p less than 5% was followed. A one-way ANOVA test was done.

RESULTS AND DISCUSSION

The results revealed significant (p<0.05) increases in the *E. coli* CFUs of the NNBM group when compared with these from the SW group. These levels decreased significantly (p<0.05) in all groups, especially in the 0T group (Figure 1).

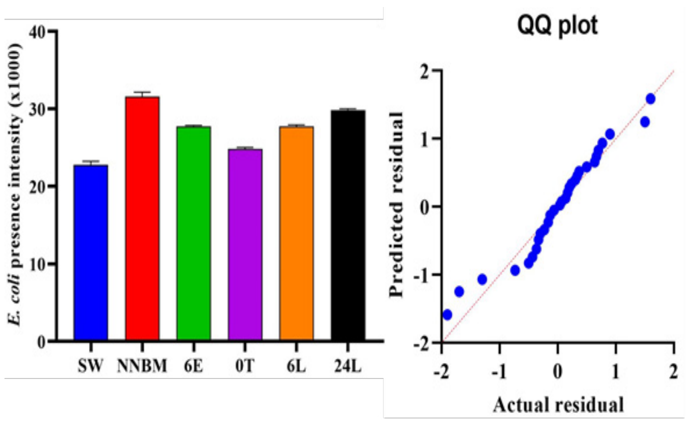

Figure 1: *E. coli* presence intensity in *Anopheles* mosquitoes after treatments with nanocurcumin.

Table 1 represents the CFU for each group with their rates.

Table 1: CFU of *E. coli* in each group.

Group	CFU (x1000)	Percentage Reduction
SW	10	67%
NNBM	30	0%
6E	25	17%
0T	20	33%
6L	22	27%
24L	30	0%

In the case of the metabolic activity, significant (p<0.05) numbers of metabolites increased in their abundance in the groups treated with NBM, such as pantothenic acid, proline, and trehalose, while a low number of compounds faced decreases in their abundance (data not shown) (Figure 2).

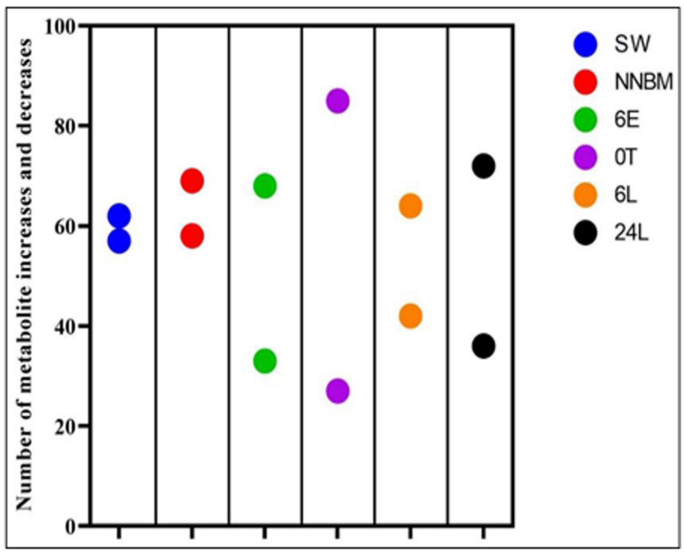

Figure 2: Numbers of metabolites increases and decreases in the presence of nanocurcumin against *E. coli* in *Anopheles* mosquitoes.

Links Researchers

In the case of the metabolic activity, significant (p<0.05) increases were seen in the abundance of pantothenic acid, proline, and trehalose in the groups treated with NBM (Figure 3).

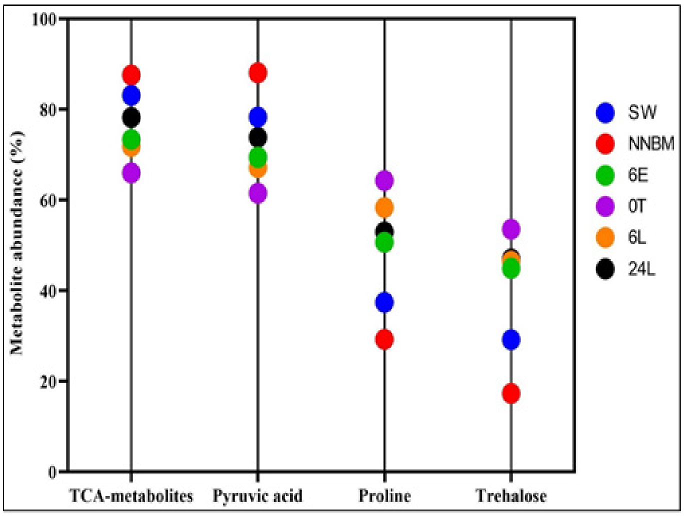

Figure 3: Abundance of metabolites in the presence of nanocurcumin against *E. coli* in the midgut of *Anopheles* mosquitoes.

The present study revealed increases in the presence percentages of *E. coli* in the exposure group; however, these increases showed declines when nanocurcumin was introduced. Curcumin is a classic medicinal substance that is frequently employed in several biological applications. Nanotechnology is being explored as a potential solution to enhance the effective utilization of curcumin. The inherent physicochemical properties of curcumin, including chemical instability, limited bioavailability, and poor water solubility, pose challenges to its pharmaceutical application. To address these limitations and enhance the therapeutic efficacy of curcumin, the utilization of nanotechnology is being investigated as a promising avenue (Chowanski *et al*., 2016; Balachandran *et al*., 2021).

A multitude of studies have shown the efficacy of curcumin in combating a diverse range of bacteria. The antimicrobial effect described in this study utilizes a multi-mechanistic approach, which involves the interaction between the nanoparticles and the membrane of the bacterium. This reaction leads to the formation of localized pores in the membrane, allowing the passage of nanoparticles into the cells of the bacteria. Once inside, these nanoparticles engage with intracellular protein molecules, thereby jeopardizing bacterial tasks (Mohanty and Sahoo., 2020). Another potential mechanism involves the attachment of nanoparticles to the microorganism membrane, followed by gradually implemented penetration into their cytoplasm and subsequent disruption of bacterial activities (Passos *et al*., 2013).

In contrast, currently employed antibiotics typically act through a limited number of mechanisms, rendering them susceptible to microbial resistance. These outcomes are consistent with previous studies by Gopal *et al*. (2016) and No *et al*. (2017), which reported that nanosized component elements exhibit greater cellular mobilization compared to their larger colleagues. The antibacterial effect was higher for the nanotechnology particle concentration of the current study than those by Othman *et al*. (2023) at (15.65-31.25 µg/mL) and by No *et al*. (2017) at (125-250 µg/mL).

In the case of the changes in the levels of metabolites, the current work revealed alterations accompanied the use of nanocurcumin. These changes may provide information related to increase resistance of mosquito digestive system to the presence of *E. coli.* This can be assured via increases in the levels of pantothenic acid, proline, and trehalose, in which these last two compounds represent fuels for a healthy mosquito instead of using glucose as a substrate and fuel the TCA cycle that could increase in an infected mosquito (Arrese and Soulages, 2010; Teulier *et al*., 2016; Giulivi *et al*., 2018; Tang *et al*., 2018; Batista *et al*., 2021; Leyria *et al*., 2021; Stec *et al*., 2021).

CONCLUSION

The current study provides important findings that nanocurcumin shows protective and treatment activities against *E. coli* load and that comes with increases in the abundance of certain important metabolites in the midgut of the mosquitoes.

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NOVELTY STATEMENT

The nanocurcumin shows protective and treatment activities against *E. coli* load.

Conflict of interest

The authors have declared no conflict of interest.

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