International Journal of Mosquito Research

ISSN: **2348-5906**
CODEN: **IJMRK2**
IJMR 2024; 11(2): 65-71

https://www.dipterajournal.com
Received: 31-12-2023
Accepted: 19-01-2024

Deepthi M
Department of Zoology, Division of Biochemistry and Molecular Biology, University of Calicut, Thenhippalam, Malappuram, Kerala, India

Kannan Vadakkadath Meethal
Department of Zoology, Division of Biochemistry and Molecular Biology, University of Calicut, Thenhippalam, Malappuram, Kerala, India

Corresponding Author:
Deepthi M
Department of Zoology, Division of Biochemistry and Molecular Biology, University of Calicut, Thenhippalam, Malappuram, Kerala, India

Trypsin modulating oostatic factor and its application as a larvicide for mosquito control: A review

Deepthi M and Kannan Vadakkadath Meethal

DOI: https://doi.org/10.22271/23487941.2024.v11.i2a.769

Abstract
Mosquitoes transmit diseases such as Dengue, Zika, Malaria, and Filariasis. Because of the development of resistance, traditional mosquito control measures are ineffective, and synthetic pesticides offer several environmental and health risks. As a result, alternative environmentally acceptable mosquito control technologies must be developed. TMOF (Trypsin Modulating Oostatic Factor) is an oostatic decapeptide, discovered in the ovaries of blood-fed female *Aedes aegypti* L. and prevents trypsin biosynthesis in their gut. TMOF isolated from *A. aegypti* (Aea-TMOF) has been shown to cause mortality in adults and larvae of several mosquito species in studies. It can be utilised as a natural bio-control agent. A comparable TMOF from the fleshfly *Neobellieria bullata* (Neb-TMOF) and another peptide, Neb-colloostatin, were discovered during the search for related folliculostatins in insects. Though chemically distinct to Aea-TMOF, the hexapeptide Neb-TMOF possesses prothoracicostatic action and is physiologically similar to Aea-TMOF. Neb-colloostatin influence egg development but do not affect trypsin biosynthesis. The gut receptor for Aea-TMOF has recently been cloned, produced, and described. In this review, we compare and contrast Aea-discovery, TMOF's structure, biosynthesis, expression in different organisms, and mosquito larvicidal action with Neb-TMOF. Finally, we talk about how TMOF and its counterparts could be used to control mosquitoes.

Keywords: Trypsin modulating oostatic factor (TMOF), folliculostatins, *Aedes aegypti*, trypsin biosynthesis, mosquito control

Introduction
Mosquitoes transmit a variety of dangerous diseases, posing a threat to both human health and the economy. Mosquito-borne diseases such as Malaria, Dengue, Chikungunya, Yellow fever, and Zika account for around 17% of all infectious diseases worldwide, claiming over 700,000 lives each year and harming millions of people's quality of life (WHO fact sheets, 2 March 2020). Malaria is spread by the Anopheline mosquito, which causes 219 million cases and over 400,000 fatalities annually, whereas Dengue fever, the most common viral infection spread by *Aedes* mosquitoes, causes 96 million symptomatic cases and 400,000 deaths annually. Furthermore, around 3.9 billion people in 129 countries are at risk of developing the disease. Dengue epidemics were only severe in 9 countries before 1970, but it is currently endemic in over 100 countries (WHO fact sheets, 19 May 2021), with Asia accounting for over 70% of the worldwide burden. The recent development of the COVID-19 pandemic may exacerbate the issue, as the combined impact of COVID-19 and Dengue fever may be even more severe, causing fatal repercussions in high-risk groups (WHO fact sheets, 19 May 2021).
Changes in environmental circumstances, travel and transportation, chaotic urbanisation, and ineffective vector control methods all contribute to an increase in the occurrence of infectious diseases. Geographical conditions, uncleanliness, and urbanisation are all factors that contribute to the growth of mosquito populations. Vector control is critical since many vector-borne diseases have no treatment options. It is a must in the case of mosquitoes due to their medical and veterinary relevance. Mosquito control has traditionally relied on chemical insecticides comprising pyrethroids, organophosphates, and other chemicals. However, this old technique increased mosquito tolerance and fostered the development of resistance.

Furthermore, environmental contamination by such compounds may cause ecological problems as well as human and cattle health risks. As a result, eco-friendly mosquito control measures are required. Biocontrol approaches for mosquito eradication have recently gained popularity since they are more environmentally friendly and have fewer non-target effects. The World Health Assembly endorsed the 'Global vector control response (GVCR) 2017-2030' in 2017 to underline the importance of vector control. The GVCR provides strategic advice for the vital strengthening of vector control in various nations and emerging partners.

Many various mosquito control tactics have been designed and used, yet mosquitoes manage to dodge them due to their tremendous adaptability. Targeting a receptor-peptide interaction used by mosquitos to regulate trypsin production is one innovative technique that has just been identified. Mosquitoes require protein-rich blood metabolites to grow their eggs. Trypsin, which is produced in the mosquito gut, aids in blood digestion. Injection of ovarian factors inhibited trypsin synthesis in arthropods, according to studies. Mosquitoes have been shown to emit ovarian factors that inhibit egg formation (Borovsky, 1985) [2]. In the mosquito gut, this oostatic factor suppresses egg formation as well as the manufacture of trypsin and chymotrypsin-like enzymes (Borovsky, 1985; 1988) [2, 3]. The name Trypsin Modulating Oostatic Factor was given to it subsequently (TMOF). Aea-TMOF is a decapeptide released by ovarian follicular cells that possess larvicidal action and were identified from *Aedes aegypti* L. (Diptera: Culicidae). Protein digestion is impeded as a result of this peptide blocking trypsin synthesis, resulting in stunted growth and mortality in the treated larvae. The peptide was isolated, the amino acid sequence was established, and the larvicidal effects of the native and mutant peptides were investigated. To test its potential as a mosquito larvicide, Aea-TMOF was also expressed in other species such as Yeast and *Chlorella*. Similar TMOF from the fleshfly *Neobellieria bullata* Park. (Diptera: Sarcophagidae) (Neb-TMOF) and another peptide, Neb-colloostatin, were discovered during a similar search for oostatic factors. Though chemically distinct from Aea-TMOF, the hexapeptide Neb-TMOF possesses prothoracicostatic action and is physiologically similar to Aea-TMOF. Egg development is likewise influenced by Neb-colloostatin, although trypsin biosynthesis is unaffected. These regulatory peptides from *Aedes aegypti* and *Neobellieria bullata* are discussed in this review, as well as the structure, production, expression in various organisms, and mosquito larvicidal action of Aea-TMOF. Finally, we talk about how TMOF and its counterparts could be used to control mosquitoes.

Oogenesis and oostatic factors in insects

The nutritional state is a major determinant of an organism's fertility. Nutritional restrictions limit fitness and, as a result, reproductive output. Multiplication, growth, and maturation of ova are all part of oogenesis in insects. The mitotic proliferation of primordial germ cells into primary oocytes occurs during the multiplication phase. The previtellogenic and vitellogenic stages are defined by the growth phase, whereas the maturation phase is defined by meiosis, which results in the conversion of the primary oocyte to the functional ovum. Insects generate centrolecithal eggs, which are yolk-rich and contain a variety of proteins, lipids, and glycogen. Vitellogenesis is a crucial step in the oogenesis process, during which vitellogenin, the yolk protein, is generated. Vitellogenin is an ovarian or extra-ovarian protein (Produced in the fat body) that is absorbed into the oocyte against a concentration gradient after being converted to vitellin, an absorbable form, under the influence of insect steroid hormones. The entry of extra ovarian proteins is assisted by pinocytosis at the oocyte membrane. Mosquitoes have polytrophic meroistic ovarioles, therefore the nurse cells give several additional macromolecules for egg formation besides vitellogenin. Oocyte multiplication and maturation take place in the ovary. A variety of changes occur in the ovary's follicular epithelium before oogenesis, including changes in patency, resorption, cell growth, and so on. Folliculostatins are peptides with a narrow range of species-specific functions (De Loof *et al*., 1995) [35]. Two of the three folliculostatins discovered in Diptera are found in the grey flesh fly *N. bullata (*Bylemans *et al*., 1993) [28], while the other is found in the mosquito *A. aegypti* (Aea-TMOF) (Borovsky *et al*., 1990, 1993) [8, 9]. In addition to ecdysone, juvenile hormone or other neuropeptides these peptides have direct or indirect role in egg development in insects.

Neobellieria bullata Folliculostatins

Trypsin modulating oostatic factor (Neb-TMOF) and Neb-colloostatin are folliculostatins in *N. bullata*. H-Asn-Pro-Thr-Asn-Leu-His-OH is the sequence of the isolated Neb-TMOF hexapeptide (Bylemans *et al*., 1994) [29]. The Neb-TMOF prevents trypsin and ecdysone biosynthesis. By blocking trypsin, it prevents food proteins from being cleaved, lowering yolk protein synthesis and thereby influencing oogenesis (Bylemans *et al*., 1994) [29]

The second folliculostatin identified from the abdomen of flesh flies, Neb-colloostatin, is a 19-mer with the sequence H-Ser-Ile-Val-Pro-Leu-Gly-Leu-Pro-Val-Pro-Ile-Glu-Pro-Ile-Val-Val-Gly-Pro-Arg-OH (Bylemans *et al*., 1995) [30]. The Neb- colloostatin inhibits yolk uptake in previtellogenic oocytes, resulting in the absence of yolk in the penultimate oocytes (Bylemans *et al*. 1995) [30]. It does not impede the synthesis of ecdysone or trypsin, unlike Neb-TMOF (Bylemans *et al*. 1995) [30].

N. bullata trypsin modulating oostatic factor (Neb-TMOF) sequence and characterization

Adult females of the larviparous grey flesh flies, *N. bullata*, require a protein meal to complete oogenesis (De Loof *et al*. 1995) [35]. In anautogenous female dipterans, the rate of trypsin activity increases to a high following a protein meal, then drops, with the amount of trypsin reaching a minimum at the conclusion of vitellogenesis (Borovsky and Schlein 1988) [3]. Neb-TMOF and its precursor are solely generated in the ovary, according to studies (Bylemans *et al*. 1996) [31]. The haemolymph of TMOF-injected test females had a reduced amount of vitellogenin on SDS-PAGE examination (Bylemans *et al*. 1994) [29]. In liver-fed females, this effect was demonstrated at physiological concentrations (10^{-9} M) of Neb-TMOF, and the three polypeptides of vitellogenin were impacted by TMOF injection (Bylemans *et al*. 1994) [29]. TMOF was isolated from the extract using HPLC, and the peptide sequence was determined using mass spectrometry (Bylemans *et al*. 1994) [29]. The peptide's amino acid sequence is NH_2-Asn-Pro-Thr-Asn-Leu-His-COOH, with the Thr being necessary for its biological activity (Bylemans *et al*. 1994) [29].

Synthetic analogues of Neb-TMOF and their activity

The activity of the hexapeptide Neb-TMOF (NH_2-Asn-Pro-Thr-Asn-Leu-His-COOH) and its analogues has been investigated. To learn more about His-6 and Asn-4, two series of analogues were produced (Konopinska *et al*. 1998) [42]. The role of Asn and His at positions 4 and 6 was investigated using two series of His (Bzl) and Phe derivatives, the second of which includes Ser, Thr, Gly, Asp, Glu, and D-Asn substitutions in position 4 (Konopinska *et al*. 1998) [42]. Para-substituted derivatives (Phe-NH_2 and Phe-NO_2) preserved biological activity, according to their findings. The activity of the nitro analogue is marginally higher than that of native Neb-TMOF, but the activity of the amino analogue is about 150 times that of native Neb-TMOF at concentrations of 10^{-8} M and substantially higher at higher concentrations (Konopinska *et al*. 1998) [42]. The inhibitory action on trypsin production of 17 analogues of the peptide was investigated via amino acid substitution and modification (Janssen *et al*. 1998) [38]. The study reveals that C-terminal replacements with lesser basic characteristics reduce biological activity, whereas residues such as lysine and para amino derivative of Phe improve inhibitory action when compared to the control. The presence of a serine residue at position 4 instead of a threonine preserves biological activity (Janssen *et al*. 1998) [38]. Comparative investigations utilising synthetic analogues show that amino acids with acidic functions like Thr-3, basic functions like His-6, and the side chains of Asn at positions 1 and 4 are necessary for the biological activity of hexapeptide (Janssen *et al*. 1998) [38].

***Aedes aegypti* trypsin modulating oostatic factor**

Reports of inhibitory activity of ovarian secretions are already discovered in dipterans. In mosquitoes, a comparable humoral substance released by the ovaries during oogenesis suppressed yolk deposition in less mature follicles (Meola and Lea 1972; Else and Judson 1972) [45, 37]. This follicle develops into an egg with the deposition of yolk after a blood meal. After oviposition, the subsequent blood meal aids in the development of the second batch of eggs. Injections of pure oostatic hormone from *Aedes* mosquitoes in females have been shown to completely block egg formation and vitellogenesis (Borovsky 1985) [2]. Injections of the *A. aegypti* oostatic hormone into *Culex quinquefasciatus*, *Culex nigripalpus*, and *Anopheles albirnanus* inhibited egg development and prevented trypsin-like enzyme synthesis in the midgut, demonstrating that the hormone is not species-specific (Borovsky 1988) [3]. *A. aegypti* Trypsin Modulating Oostatic Factor (Aea-TMOF) was called after the oostatic factor isolated from the ovaries of *A. aegypti* (Borovsky *et al*. 1990) [8].

Aea-TMOF biosynthesis and characterisation

TMOF is secreted into the haemolymph of mosquitoes following a blood meal. TMOF is not a neuropeptide, but rather an ovarian factor, according to research on the site of its manufacture. TMOF is produced by the ovarian follicular epithelium and is accumulated 18-48 hours following a blood meal (Borovsky *et al*. 1994) [29]. The peptide binds to a receptor on midgut epithelial cells, inhibiting trypsin-like enzyme production. The gut specific receptor of trypsin regulating oostatic factoris is ABCtmfA transporter which import the former into epithelial cells and the ultimate receptor is ribosomes which interfere in translation of trypsin mRNA transcript (Borovsky *et al*., 2022) [21]. TMOF concentration in the ovary does not increase significantly for 3-18 hours after a blood meal but increases rapidly to nearly twenty-fold for 18-48 hours. Following that, as the ovary matured and the follicular epithelium stretched and thinned, the concentration decreased (Borovsky *et al*. 1994) [29]. The midgut of *A. aegypti* was removed at intervals to study trypsin biosynthesis in the midgut (Borovsky and Schlein 1988) [22]. The rate of synthesis of trypsin-like enzymes in the midgut is reported to increase 24 hours after a blood meal and then fall to a low level 48 hours later. The TMOF synthesis was then connected with this. The highest synthesis of TMOF in the ovary is inversely related to trypsin synthesis, which is observed 36 hours after the blood meal (Borovsky *et al*. 1994b) [29]. TMOF has a half-life of 1.6 hours, according to experiments utilising ^{3}H (^{3}H-TMOF) (Borovsky *et al*. 1993) [28].

Because of its oostatic effect, the TMOF decapeptide has been extensively researched. The peptide's primary sequence is Tyr-Asp-Pro-Ala-Pro-Pro-Pro-Pro-Pro-Pro, with an atomic mass of 1047.6 Da (Borovsky *et al*. 1990) [8]. The solution structure of TMOF was investigated using 2-D ^{1}H NMR spectroscopy and molecular modelling (Curto *et al*. 1993) [33]. It does not, however, disrupt the peptide helix's left-handed orientation, which is produced by 3-10 Pro residues (Curto *et al*. 1993) [33]. The peptide was discovered to be a 30 Å long rod-shaped left-handed helix with no β turns (Curto *et al*. 1993) [33].

Structural analogues of TMOF and their activity

Although the action of Aea-TMOF is not species-specific (Borovsky 1988) [2], there are changes in the peptide's efficacy in different mosquito species, which could be related to the presence of different analogues of the same in different species (Borovsky and Meola 2004) [15] or receptor variation. To determine the rationale for the aforementioned observation, the structure of TMOF and the receptor from different mosquito species must be fully elucidated. There are six Pro residues at the C-terminus of TMOF (Tyr-Asp-Pro-Ala-Pro-Pro-Pro-Pro-Pro-Pro), forming a left-handed helix (Borovsky *et al*. 1990, 1993; Curto *et al*. 1993) [8-9, 33]. TMOF activity is reduced when Pro residues are removed from the C terminus of the peptide (Tyr-Asp-Pro-Ala-Pro-Pro-Pro, Tyr-Asp-Pro-Ala-Pro-Pro and Tyr-Asp-Pro-Ala-Pro). The addition of Arg at the end (Tyr-Asp-Pro-Ala-Pro-Arg) reduces activity by 1.2 times, while the activity of the analogue is lost when Lys is replaced with Arg (Tyr-Asp-Pro-Ala-Pro-Lys). TMOF activity is retained at 95 percent by the tetrapeptide Tyr-Asp-Pro-Ala alone, and activity is increased by 1.6 fold by adding Arg to the C-terminus (Borovsky and Meola 2004) [15]. The activity of the Asp-Pro-Ala-Arg analogue is lower than that of Asp-Pro-Ala, although it has increased significantly for $(Asp-Pro-Ala-Arg)_4$. The tri-peptide Tyr-Asp-Pro lost around 91% of its activity, although d-amino acid replacements enhanced activity by 39% for (d)Tyr-Asp-Pro and 71% for Tyr-(d)Asp-Pro (Borovsky and Meola 2004) [15]. The active core for gut receptor binding is found at the N-terminus, and the shortest peptide for efficient receptor binding is Tyr-Asp-Pro-Ala, according to studies on 29 TMOF analogues (Borovsky and Meola 2004) [15]. The TMOF and its analogues (Aromatic and aliphatic organic acids, as well as ester analogues) were examined in *A. aegypti*, *Heliothis virescens*, and *Plutella xylostella*, the diamondback moth. Except for the natural

TMOF, these chemicals caused larval death in *A. aegypti* and *P. xylostella*, but not in *H. virescens* (Borovsky and Nauen 2007) [16]. The addition of Lys to the C-terminus of TMOF (TMOF-Lys) reduces its insecticidal action, but aliphatic PEGylation at Lys (TMOF-Lys-PEG_7P) boosted insecticidal activity against *A. aegypti* and *A. albopictus* larvae by around 3.3 times over TMOF (Jeffers *et al*. 2012) [39].

Trypsin modulating oostatic factor as a larvicide

In mosquito larvae, trypsin-like enzymes is physiologically crucial for digestion (Yang and Davies 1971) [49]. The digestive enzymes trypsin and chymotrypsin grow from the first to the fourth instar, then decreases as the larva undergoes pupation (Yang and Davies 1971) [49]. The use of TMOF in mosquito control was investigated because of the role of trypsin in mosquito larvae. TMOF and its equivalents were found to have a larvicidal effect when feeding larvae along with Brewer's yeast. It was confirmed that the amount of TMOF fed to the larvae had an inverse relationship with their survival and that the tetrapeptide Tyr-Asp-Pro-Ala alone is sufficient to retain 95% of TMOF activity (Borovsky and Meola 2004) [15]. TMOF was also supplied to the larvae as yeast adsorbed virions after being produced as a fusion protein with the coat protein of the tobacco mosaic virus. This resulted in a 90% suppression of trypsin activity, which resulted in larval starvation and death (Borovsky *et al*. 2006) [20]. When mosquito larvae were fed TMOF expressed in *Pichia pastoris* cells, it had a synergistic impact with Cry proteins (Borovsky *et al*. 2010) [13]. Increased virulence against larvae and adults of *A. gambiae* caused mortality and reduced fecundity when Aea-TMOF was expressed in the *Beauveria bassiana*, an entomopathogenic fungus (Kamareddine *et al*. 2013) [41]. Genetically modified *Chlorella desiccata* with the *tmf*A gene for producing TMOF peptide fed to *A. aegypti* larvae resulted in limited survival and caused mortality of up to 96 percent (Borovsky 2014; Borovsky *et al*. 2016) [6, 26]. Heat-killed *Saccharomyces cerevisiae* cells transformed with gene, *tmf*A and gfp-*tmf*A produced 95 percent mortality in *A. aegypti* (Borovsky *et al*. 2018) [17]. Multiple insertions of the *tmf*A gene were performed in *P. pastoris* cells using a multi-copy integrating plasmid pICZB, resulting in greater expression of TMOF higher mortality of 88 percent within three days (Borovsky *et al*. 2020) [18].

Toxicity of TMOF to non-target organisms

TMOF could be utilised as a biorational pesticide, according to the researchers (Borovsky 2003) [4]. The biological activity of this decapeptide has been investigated in adults and larval mosquitos, with the idea of utilising it as a biorational insecticide being considered (Borovsky *et al*. 1990, 1991, 1993; Borovsky and Meola 2004) [8, 10, 9, 15]. Any pesticide's non-target effect is a hazard to the environment. TMOF's impact on other arthropods and vertebrates has been investigated. Injections of Aea-TMOF and its synthetic analogues inhibited the manufacture of trypsin-like enzymes in Dipteran flies such as *Stomoxys calcitrans*, *M. domestica*, and *Culicoides variipennis*, as well as the Siphonapteran flea *Ctenocephalides felis* (Borovsky *et al*. 1990) [8]. Injections of Aea-TMOF have been shown to have insecticidal action against *Heliothis virescens* (Nauen *et al*. 2001) [47], *Anthonomas grandis* (Borovsky 2007) [16]. On fed transgenic tobacco leaves expressing the gene for (Arg-TMOF-Arg)n, the tobacco budworm, *H. virescens*, showed retarded growth and development (Tortiglione *et al*. 2002) [48]. In addition, an in-vitro assay using the peptide revealed a significant inhibitory effect on serine protease production as compared to controls in this study. After a 12-day exposure to escalating TMOF concentrations, the freshwater prawn *Macrobrachium rosenbergii* was found to have significantly decreased survival and growth (Dauda *et al*. 2016) [34]. The arthropod crustacean *Daphnia*, on the other hand, has never been linked to toxicity (Thompson *et al*. 2004) [36]. When TMOF was tested on higher vertebrates such as mice, mallard ducks, and rabbits, no toxicity was found. The reason for this, according to Thompson *et al*. (Thompson *et al*. 2004) [36], is because TMOF is degraded by vertebrate proteases like leucine aminopeptidases. Despite containing aminopeptidases, *M. rosenbergii* exposed to TMOF has a decreased survival rate. As a result, the decrease of toxicity may not be entirely due to TMOF degradation; the presence or lack of the receptor, as well as its relative abundance, may all have a role in the toxicity. This is something that needs to be looked at. Small molecule agonists of the TMOF gut receptor can be identified using small molecule screening, which will aid in the development of better mosquito larvicide.

Synergism of TMOF with other insecticidal proteins

Aea-TMOF and its equivalents have been evaluated individually as well as in combination formulations against *A. aegypti*. *Bacillus thuringiensis* subspecies *israeliensis* (*Bti*) is a biological control agent for mosquito larvae and black fly larvae (Ben-Dov 2014) [1]. TMOF and *Bti* were combined in various formulations and tested against mosquito larvae. The activity of TMOF with *Bti* rice husk formulations, TMOF and *Bti* wettable powder, TMOF and *Bti* mosquito fudge cubes, recombinant TMOF yeast cell paste form and recombinant yeast cell dried powder against *A. aegypti* larvae were investigated (Misni *et al*. 2010) [46]. According to the findings, *Bti* rice husk and wettable powder caused complete mortality from 24 hours to 4 weeks after exposure, whereas mosquito fudge cubes caused substantial mortality from the first to the 12th week after exposure. Pichia-TMOF has a larvicidal action, while *Bti* and Pichia-TMOF have a synergistic impact on *A. aegypti* (Lau *et al*. 2011) [43]. Feeding *A. aegypti* larvae with *P. pastoris* and *Escherichia coli* expressing the *tmf*A and *Bti* toxin genes, respectively, revealed synergistic actions of TMOF and Cry proteins from *Bti* (Borovsky *et al*. 2010) [13]. *P. pastoris* expressing the fusion protein of TMOF and Cry protein (cry4Aa-*tmf*A) caused about 90% mortality in *A. aegypti* larvae (Borovsky *et al*. 2011) [19].

Conclusions

Mosquitoes are carriers of a variety of viral or protozoan diseases. As there is no cure for the viral diseases, mosquito management is critical for preventing the spread of mosquito-borne diseases. The chemical method of mosquito control by use of synthetic insecticides is an important and widely used control strategy. The health hazards posed by synthetic insecticide to humans and other animals, as well as the development of resistance to insecticide are some of the severe challenges that must be addressed. Thus an environment-friendly mosquito control method is urgently required. The use of an ovarian peptide called Trypsin modulating oostatic factor (TMOF) is one of such alternatives. The TMOF decapeptide (Tyr-Asp-Pro-Ala-Pro-Pro-Pro-Pro-Pro-Pro), is isolated from the ovaries of blood-

fed *A. aegypti* mosquitoes and it stops digestion and causes mortality by inhibiting the formation of trypsin-like enzymes in the midgut. *Neobellieria*-TMOF is a functionally similar hexapeptide (Asn-Pro-Thr-Asn-Leu-His) obtained from the fleshfly *N. bullata* (Neb-TMOF). This peptide prevents trypsin and 20-hydroxyecdysterone from being produced. This prevents the synthesis of yolk proteins and the uptake of yolks. Neb-colloostatin from *N. bullata* is another peptide that inhibits yolk uptake in previtellogenic oocytes. The amino acid residues Thr-3, His-6, and Asn-1 plays important role in the biological activity of Neb-TMOF. The *A. aegypti* trypsin modulating oostatic factor (Aea-TMOF) is a decapeptide with a left-handed helical shape. It hinders adult females from digesting blood, causing delay in egg development. The TMOF decapeptide has a half-life of 1.6 hours and works by interacting with a midgut receptor with a 1307-amino-acid protein that is recently identified. Using several structural analogues of TMOF, generated by eliminating amino acid residues or changing the sequence, it was found that the tetrapeptide, Tyr-Asp-Pro-Ala, is sufficient to preserve roughly 95% of TMOF activity. As a larvicide, the decapeptide is found to be equally effective in the larvae of mosquitoes. The peptide's effectiveness in mosquito larvae led to it being used as a biorational pesticide for mosquito control. Trypsin modulating oostatic factor has been effectively expressed in *P. pastoris*, *B. bassiana*, *C. desiccata*, and *S. cerevisiae*. When these organisms producing the peptide were fed to mosquito larvae, they caused significant mortality even up to 100% in certain cases. The TMOF from *A. aegypti* is also efficient against *Culex* and *Anopheles* mosquitoes, indicating that it acts across species. The synergistic effect of TMOF's with other bacterial toxins, such as *Bt* toxin, has also been demonstrated. When conjugated with other macromolecules, such as PEG, the activity of TMOF increases. The TMOF isolated from *A. aegypti* was reported to suppress trypsin production in *S. calcitrans*, *M. domestica*, *H. virescens*, *P.xylostella*, *C. felis*, and *C.variipenis*, in addition to mosquitoes. The widespread activity could be explained by the presence of similar receptors in these organisms. The efficacy depends on the relative level of expression of the gut receptors and affinity of the peptide for the receptor. By employing the molecular docking it is possible to identify better small molecule agonists for the receptor from synthetic chemical library or plant compound library. Toxicity to TMOF has not been reported in crustaceans like *Daphnia* or vertebrates like mice, mallard ducks, or rabbits. The non-responsiveness could be attributed to a lack of TMOF receptors in these organisms. Also proteases such as leucine aminopeptidases have been shown to degrade TMOF in vertebrates. Despite the presence of leucine aminopeptidases, the peptide triggers a lower survival rate in *M. rosenbergii* when exposed to increasing concentrations of TMOF. Identification of better molecules which can mimic TMOF and with increased receptor affinity will result in a more effective pesticide. Non-target effects can also be decreased by discovering analogues that are more specific to the receptors of specific organism.

Acknowledgements
The authors would like to thank Department of Zoology, University of Calicut for the infrastructure facilities. We also thank Council of Scientific & Industrial Research, India for financial support of the author Deepthi M, in the form of CSIR Junior Research Fellowship.

Availability of Data and Material: Not applicable.

Ethics Approval: This paper does not contain any studies neither with human participants nor with animals.

Conflict of interest declaration: The authors declared none.

References

1. Ben-Dov E. Bacillus thuingiensis subsp. Israeliensis and its dipteran specific toxins. Toxins (Basel). 2014;6(4):1222-1243. https://doi.org/10.3390/toxins6041222
2. Borovsky D. Isolation and characterization of highly purified mosquito oostatic hormone. Arch Insect Biochem Physiol. 1985;2(4):333-349.
3. Borovsky D. Oostatic hormone inhibits biosynthesis of midgut proteolytic enzymes and egg development in mosquitoes. Arch Insect Biochem Physiol. 1988;7(3):187-210. https://doi.org/10.1002/arch.940070305
4. Borovsky D. Trypsin modulating Oostatic factor: A potential new larvicide for mosquito control. J Exp. Biol. 2003;206:3869-3875.
5. Borovsky D. Trypsin Modulating Oostatic Factor for developing resistant crops. In: Ishaaya I, Nauen R, Horowitz R, eds. Insecticides Design Using Advanced Technologies. Springer-Verlag; 2007:135-149.
6. Borovsky D. Trypsin Modulating Oostatic Factor (TMOF) and Insect Biotechnology. In: Hoffmann K, ed. Insect Molecular Biology and Ecology. CRC Press; 2014:318-349.
7. Borovsky D, Stberner A, Powell CA. Cloning and expressing Trypsin Modulating Oostatic Factor in Chlorella desiccata to control mosquito larvae. Arch Insect Biochem Physiol. 2016;91:17-36. https://doi.org/10.1002/arch.21306
8. Borovsky D, Carlson DA, Griffin PR, Shabanowitz J, Hunt DF. Mosquito oostatic factor: a novel decapeptide modulating trypsin like enzyme biosynthesis in the midgut. Faseb J. 1990;4(12):3015-3020. https://doi.org/10.1096/fasebj.4.12.2394318
9. Borovsky D, Carlson DA, Griffin PR, Shabanowitz J, Hunt DF. Mass spectrometry and characterization of *Aedes aegypti* trypsin modulating oostatic factor (TMOF) and its analogs. Insect Biochem Mol. Biol. 1993;23(6):703-712. https://doi.org/10.1016/0965-1748(93)90044-S
10. Borovsky D, Carlson DA, Hunt DF. Mosquito oostatic hormone: A trypsin modulating oostatic factor. In: Menn JJ, Kelly TJ, Masler EP, eds. Insect Neuropeptides Chemistry, Biology and Action. 1991:133-142.
11. Borovsky D, Carlson DA, Ujvfiry I. *In vivo* and *in vitro* biosynthesis and metabolism of methyl farnesoate, juvenile hormone III and juvenile hormone III acid in the mosquito *Aedes aegypti*. J Med. Entomol. 1992;29(4):619-629. https://doi.org/10.1093/jmedent/29.4.619
12. Borovsky D, Deckers K, Vanhove AC, *et al*. Cloning and characterization of *Aedes aegypti* Trypsin Modulating Oostatic Factor (TMOF) gut receptor. Biomolecules. 2021;11:934. https://doi.org/10.3390/biom11070934

13. Borovsky D, Khasdan V, Nauwelaers S, *et al*. Synergy between *Aedes aegypti* Trypsin Modulating Oostatic Factor and δ-Endotoxins. T O Toxi J. 2010;3:141-150. https://doi.org/10.2174/1875414701003010141
14. Borovsky D, Mahmood F. Feeding the mosquito *Aedes aegypti* with TMOF and its analogs, effect on trypsin biosynthesis and egg development. Regul. Pept. 1995;57(3):273-281.
15. Borovsky D, Meola SM. Biochemical and cytoimmunological evidence for the control of *Aedes aegypti* larva trypsin with Aea-TMOF. Arch Insect Biochem Physiol. 2004;55(3):124-139. https://doi.org/10.1002/arch.10132
16. Borovsky D, Nauen R. Biological and biochemical effects of organo-synthetic analogues of Trypsin Modulating Oostatic Factor (TMOF) on *Aedes aegypti*, Heliothis virescens and *Plutella xylostella*. Pestycydy. 2007;(3-4):17-26.
17. Borovsky D, Nauwelaers S, Powell CA, Shatters RG Jr. Cloning, Genetic Engineering and Characterization of TMOF expressed in Saccharomyces cerevisiae to control larval mosquitoes. J Insect Physiol. 2018;106(2):134-146. https://doi.org/10.1016/j.jinsphys.2017.01.008
18. Borovsky D, Nauwelaers S, Shatters R Jr. Biochemical and molecular characterization of *Pichia pastoris* cells expressing multiple TMOF genes (tmfA) for mosquito larval control. Front Physiol. 2020;11:527. https://doi.org/10.3389/fphys.2020.00527
19. Borovsky D, Nauwelaers S, Van Mileghem A, *et al*. Control of mosquito larvae with TMOF and 60 kDa Cry4Aa expressed in *Pichia pastoris*. Pestycyde/Pesticides. 2011;1-4:5-15.
20. Borovsky D, Rabindran S, Dawson WO, *et al*. Expression of Aedes trypsin-modulating oostatic factor on the virion of TMV: A potential larvicide. P Natl. Acad. Sci. USA. 2006;103(50):18963-18968. https://doi.org/10.1073/pnas.0606146103
21. Borovsky D, Rouge P, Shatters RG Jr. The Ribosome is the ultimate receptor for trypsin modulating Oostatic factor (TMOF) Biomol. 2022;12(4):577.
22. Borovsky D, Schlein Y. Quantitative determination of trypsin-like and chymotrypsin-like enzymes in insects. Arch Insect Biochem Physiol. 1988;8(4):249-260. https://doi.org/10.1002/arch.940080406
25. Borovsky D, Song Q, Ma M, Carlson DA. Biosynthesis, secretion and cytoimmunochemistry of trypsin modulating oostatic factor of *Aedes aegypti*. Arch Insect Biochem Physiol. 1994;27(1):27-38. https://doi.org/10.1002/arch.940270105
26. Borovsky D, Sterner A, Powell CA. Cloning and expressing trypsin modulating oostatic factor in Chlorella desiccata to control mosquito larvae. Arch Insect Biochem Physiol. 2016;91(1):17-36. https://doi.org/10.1002/arch.21306
27. Borovsky D, Thomas BR, Carlson DA, Whisenton LR, Fuchs MS. Juvenile hormone and 20-hydroxyecdysone as primary and secondary stimuli of vitellogenesis in *Aedes aegypti*. Arch Insect Biochem Physiol. 1985;2(1):75-90. https://doi.org/10.1002/arch.940020108
28. Bylemans D, Borovsky D, De Loof A. Partial characterization of two trypsin modulating oostatic factors from the ovaries of *Sarcophaga bullata*, the grey flesh fly. In: Borovsky D, Spielman A, eds. Host Regulated Developmental Mechanisms in Vector Arthropods. Proceedings of the third Symposium, University of Florida; c1993. p. 09-25.
29. Bylemans D, Borovsky D, Hunt DF, Shabanowitz J, Grauwels L, De Loof A. Sequencing and characterization of trypsin modulating oostatic factor (TMOF) from the ovaries of the grey flesh fly, Neobellieria (Sarcophaga) bullata. Regul. Pept. 1994;50(1):61-72. https://doi.org/10.1016/0167-0115(94)90192-9
30. Bylemans D, Proost P, Samijn B, Borovsky D, Grauwels L, Huybrechts R, *et al*. Neb-colloostatin, a second folliculostatin of the grey flesh fly, *Neobellieria bullata*. Eur J Biochem. 1995;228(1):45-49.
31. Bylemans D, Verhaert P, Janssen I, Vanden Broeck J, Borovsky D, Ma M, *et al*. Immunolocalizaion of the oostatic and prothoracicostatic peptide, Neb-TMOF, in adults of the flesh fly, *Neobellieria bullata*. Gen Comp Endocrinol. 1996;103(3):273-280. https://doi.org/10.1006/gcen.1996.0122
32. Clements AN. The Biology of Mosquitoes. New York: Chapman & Hall; c1992.
33. Curto EV, Jarpe MA, Blalock JB, Borovsky D, Krishna NR. Solution structure of trypsin modulating oostatic factor is a left-handed helix. Biochem Biophys Res Commun. 1993;193:688-693. https://doi.org/10.1006/bbrc.1993.1679
34. Dauda AB, Teh JC, Amin SMN, Kamarudin MS, Romano N. Trypsin-modulating oostatic factor (TMOF) decreased the survival, growth and digestion enzymes of Macrobrachium rosenbergii. J Pestic. Sci. 2016;41(3):83-86. DOI:10.1584/jpestics.D16-055
35. De Loof A, Bylemans D, Schoofs L, Janssen L, Huybrechts R. The folliculostatins of two dipteran insect species, their relation to matrix proteins and prospects for practical applications. Entomol Exp. Appl. 1995;77:01-09.
36. Thompson DM, Young HP, Frank WE, *et al*. Non-target toxicology of a new mosquito larvicide, trypsin modulating oostatic factor. Pestic. Biochem Phys. 2004;80(3):131–142. DOI:10.1016/j.pestbp.2004.06.009
37. Else JG, Judson CL. Enforced egg-retention and its effect on vitellogenesis in the mosquito *Aedes aegypti*. J Med Entomol. 1972;9:527-530.
38. Janssen I, Koolman J, Konopinska D, Bartosz-Bechowski H, Schoofs L, De Loof A. Biological activity of structural analogs and effect of oil as a carrier of trypsin modulating oostatic factor of grey flesh fly *Neobellieria bullata*. Peptides. 1998;19:627-634.
39. Jeffers LA, Shen H, Khalil S, *et al*. Enhanced activity of an insecticidal protein, trypsin modulating oostatic factor (TMOF), through conjugation with aliphatic polyethylene glycol. Pest Manag Sci. 2012;68(1):49-59. DOI:10.1002/ps.2219
40. Judson CL. Physiology of feeding and oviposition behavior in *Aedes aegypti* (L.). J Med. Entomol. 1968;5:21-23. doi:10.1093/jmedent/5.1.21
41. Kamareddine L, Fan Y, Osta MA, Keyhani NO. Expression of trypsin modulating oostatic factor (TMOF) in an entomopathogenic fungus increases its virulence towards Anopheles gambiae and reduces fecundity in the target mosquito. Parasite Vector. 2013;6:22. DOI:10.1186/1756-3305-6-22
42. Konopinska D, Bartosz-Bechowski H, Kuczer M, *et al*.

Insect trypsin modulating oostatic factor (Neb-TMOF) and its analogs: preliminary structure/biological function relationship studies. Lett. Pept. Sci. 1998;5:391-393.
43. Lau YS, Sulaiman S, Othman H. The effectiveness of trypsin modulating oostatic factor (TMOF) and combination of TMOF with Bacillus thuringiensis against *Aedes aegypti* larvae in the laboratory. Iran J Arthropod-bor. 2011;5(1):13-19.
44. Liu TP, Davey KG. Partial characterization of a proposed antigonadotropin from the ovaries of the insect *Rhodnius prolixus* Stal. Gen Comp Endocrinol. 1974;24:405-408.
45. Meola R, Lea AO. Humoral inhibition of egg development in mosquitoes. J Med. Entomol. 1972;9:99-103. DOI:10.1093/jmedent/9.1.99
46. Misni N, Othman HF, Sulaiman S. Larvicidal effect on trypsin modulating oostatic factor (TMOF) formulations on *Aedes aegypti* larvae in the laboratory. J Trop. Med. Parasitol. 2010;33:69-76.
47. Nauen R, Sorge D, Sterner A, Borovsky D. TMOF like factor controls the biosynthesis of serine proteases in the larval gut of Heliothis virescens. Arch Insect Biochem Physiol. 2001;47(4):169-180. DOI:10.1002/arch.1049
48. Tortiglione C, Fanti P, Pennacchio F, *et al*. The expression in tobacco plants of *Aedes aegypti* trypsin modulating oostatic factor (Aea-TMOF) alters growth and development of the tobacco budworm, Heliothis virescens. Molec. Breeding. 2002;9:159-169. DOI:10.1073/pnas.0606146103
49. Yang YJ, Davies DM. Trypsin and chymotrypsin during metamorphosis in *Aedes aegypti* and properties of the chymotrypsin. J Insect Physiol. 1971;17:117-131. DOI:10.1016/0022-1910(71)90048-5