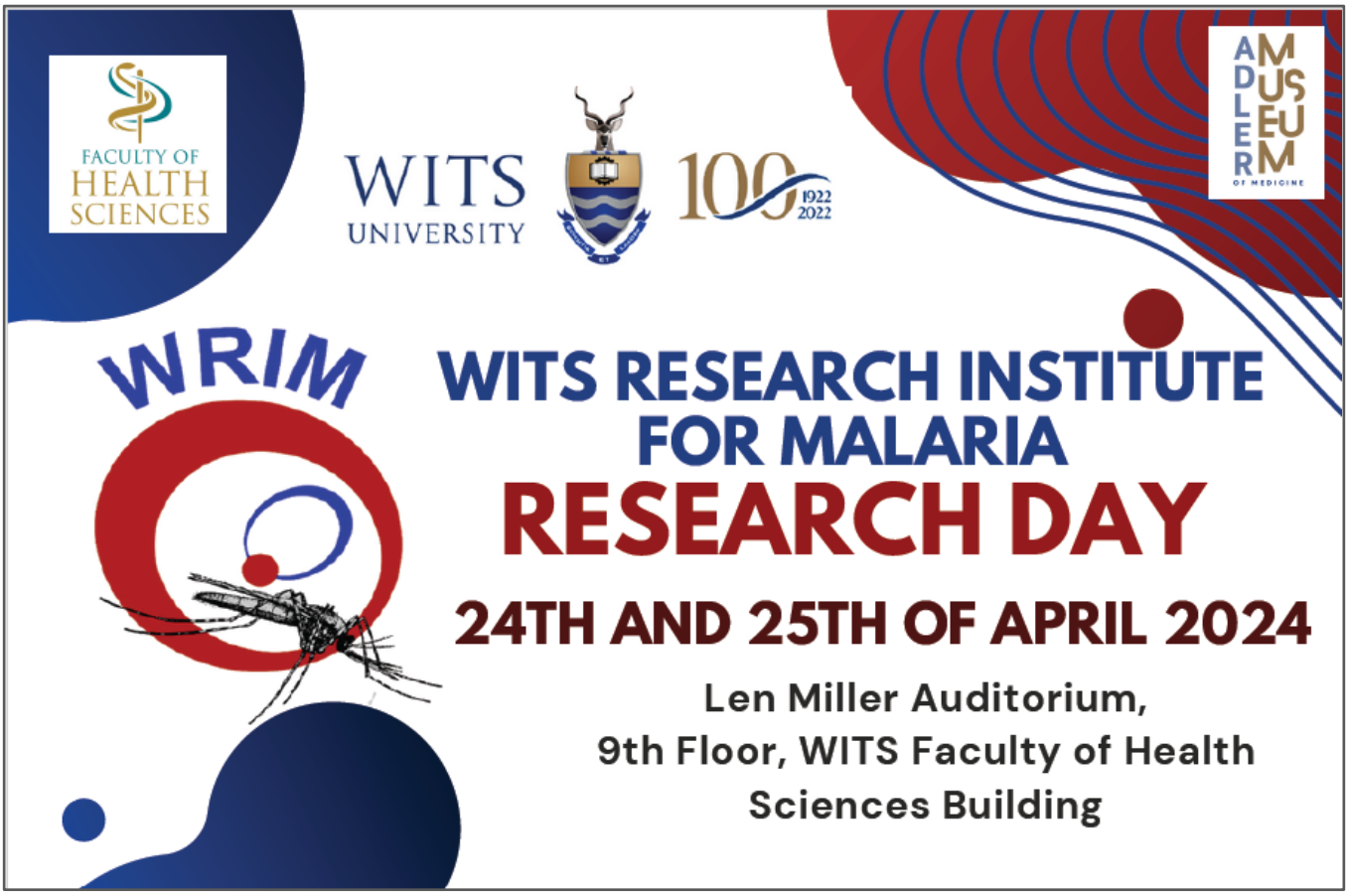


ABSTRACT BOOKLET

AND

PROGRAMME

MESSAGE FROM CO-DIRECTORS

Dear esteemed colleagues,

It is a momentous occasion as we gather for our second Research Institute for Malaria Research Day, a crucial platform to share and advance our knowledge. This year, we also have the privilege to unite with others worldwide in celebrating World Malaria Day, a testament to our shared commitment to 'accelerate the fight against malaria for a more equitable world'.

I extend my sincerest congratulations to each of you for graciously sparing time from your demanding schedules to participate in this event. The abundance of abstract submissions is a testament to our collective dedication, and I am particularly grateful to Professor Robyn van Zyl and her team for the valuable contributions they made to organise this occasion in such a short time. As we engage in dialogue and exchange ideas throughout the day, let us remain cognisant of the significance of your collective endeavours to alleviate the burden of malaria directly or indirectly.

In closing, we extend our heartfelt gratitude to each institute member for their unwavering dedication and commitment. May today's deliberations inspire new insights and collaborations, driving us closer to our ultimate goal of eradicating malaria.

With warm regards,

Robyn and Lizette


24th April 2024

Teams On-Line - Click here to join the meeting https://shorturl.at/csyFU
Meeting ID: 377 908 849 514 Passcode: RY5Su7

CATEGORY	SESSION CHAIR	TIME	PRESENTER	TITLE
Welcome	Prof Lizette Koekemoer	15:30-15:35	**Prof Robyn van Zyl**	Welcome to Day 1 on online WRIM Postgraduate Research Day 2024
Plenary	Prof Lizette Koekemoer	15:35 – 16:15	**Prof Tony Nolan**	"Controlling mosquito populations genetically"
End of Day 1	Jabu Mahlangu	16:15-16:30	**Prof Robyn van Zyl**	Until tomorrow when we meet in person ….

25th April 2024

WITS Faculty of Health Science Building, Len Miller Theater, 9th floor

CATEGORY	SESSION CHAIR	TIME	PRESENTER	TITLE
Registration		8:00 – 9:00		Registration with Tea and Coffee
Welcome	**Prof Robyn van Zyl**	9:00 – 9:15	**Prof Lindelani Mnguni**	Welcome to Day 2 of in-person WRIM Postgraduate Research Day 2024
Life-Time Achievement Award	**Prof Lizette Koekemoer**	9:15 – 9:45	**Prof Miles Markus**	"Hidden parasites in malaria" **Join the meeting now** httQs://s ho rtu rl.at/km rYZ Meeting ID: 376 520 538 490; Passcode: 3ZqbZw
Housekeeping	**Mr Jabu Mahlangu**	9:45 – 10:00	**Ms Huang-miao Chen**	To note….

CATEGORY	SESSION CHAIR	TIME	PRESENTER	TITLE
Session One	**Dr Yael Dahan-Moss**	10:00 – 10:15 (PostDoc)	**Burke, Ashley**	Biting Back: screening the MMV Global Health Priority Box for endectocidal activity against malaria vector mosquitoes
		10:15 – 10:30 (MSc, 15min)	**Misser, Shristi**	The detection and effect of microplastics on the gut microbiota of the major malaria vector, *Anopheles arabiensis* Patton (Diptera: Culicidae)
		10:30 –10:35 (MSc, 5x5)	**Malan, Sune**	The properties of 4-allyl-2-methoxyphenol derivatives as possible larvicides and metal chelators
		10:35 – 10:37 (MSc, 2x2)	**Dennis, Lauren**	Knowledge, attitudes, and practices of malaria management in the Ehlanzeni District, Mpumalanga, South Africa
		10:37 - 10:40	**All in session**	Open question session
Tea break	20 min	10:40 – 11:00	**WRIM RD Marketing Team slide show**	
Session Two	**Mr Jabu Mahlangu**	11:00-11:15 (PostDoc)	**Chen, Chia-Yu**	The effect of immune stimulation on histone modification of the major malaria vector *Anopheles arabiensis patton* (Diptera: Culicidae)
		11:15 – 11:30 (PhD, 15min)	**Bloch, Nerissa**	Heat shock factor (HSF) expression and its effect on life table parameters in the main African malaria vector *Anopheles funestus*
		11:30 – 11:45 (MSc, 15min)	**Mazarire, Theresa**	The impact of climatic factors on temporal mosquito distribution and population dynamics in an area targeted for Sterile Insect Technique pilot trials
		11:45 – 11:50 (PhD, 5x5)	**Jamesboy, Eunice**	Unlocking History: Assessing DNA Extraction Kits for Ancient *Anopheles* Specimens in the Museums
		11:50 - 11:55 (Hons, 5x5)	**Machweu, Thato**	Assessing potential bottlenecks in the mass production of sterilised male *Anopheles arabiensis* for a South African sit feasibility study
		11:55 –11:57 (MSc, 2x2)	**Rakgoroana, Mogale**	The repellent effects of naturally derived essential oils against the anopheline mosquitoes
		11:57-12:00	**All in session**	Open question session
Time-Out	**Ms M Kalonji**	12:00 – 12:05		.... Buzz like a mosquito ....
Session Three	**Ms Avhatakali Matamba**	12:05 – 12:20 (PostDoc)	**Makumbe, Hattie**	Variable effects of urban and rural pollutants on the life history of the major malaria vector *Anopheles arabiensis* (Diptera: Culicidae),
		12:20 – 12:35 (PhD, 15min)	**Singh, Ashmika**	The dynamic gut microbiota of laboratory reared, the first filial population, and wild population of the major malaria vector *Anopheles arabiensis* Patton (Diptera: Culicidae)
		12:35 –12:50 (MSc, 15min)	**Hape, Emmanuel**	Mating in captivity: An observational study of copulation events between *Anopheles arabiensis* and *An. funestus*
		12:50 –12:55 (MSc, 5x5)	**Chiliza, Unathi**	HPLC method development and validation of (-)-alpha-pinene
		12:55 –13:00	**All in session**	Open question session
Lunch	45 min	13:00 – 13:45	**WRIM RD Marketing Team slide show**	

CATEGORY	SESSION CHAIR	TIME	PRESENTER	TITLE
Session Four	**Dr Armorel van Eyk**	13:45 – 14:00 (PhD, 15min)	**Agarwal, Priyanka**	Design, molecular docking, drug-likeness and ADMET prediction of chalcones as novel *Plasmodium falciparum* cytochrome bc1 (cyt bc1) inhibitors
		14:00-14:15 (PhD, 15min)	**Kalonji, Mbuyi**	Exploring the potential of using the sugar water diet to enhance *An. funestus* laboratory colonisation
		14:15 –14:20 (MSc, 5x5)	**Samuel, Michael**	Analysis of a 3d-printed irradiation canister for bulk *Anopheles arabiensis* sterilization in a South African sterile insect technique
		14:20 –14:25 (MSc, 5x5)	**Govender, Venisha**	The inference of OTC phytomedicines on oxidative stress, metal homeostasis, and *Plasmodium falciparum* survival
		14:25 –14:30	**All in session**	Open question session
Time-Out	**WSPS**	14:30 – 14:35		…. Wiggle like a merozoite ….
Session Five	**Dr Maria Kaiser**	14:35 – 14:50 (PostDoc)	**Medjigbodo, Adandé**	Impact of insecticide exposure on *Plasmodium falciparum* development in resistant *An. gambiae* mosquitoes
		14:50–15:05 (PhD, 15min)	**Makhulu, Edward**	A fight for dominance: *Microsporidia MB* abundance and intensity in germline and somatic tissues
		15:05 –15:20 (PhD, 15min)	**Zengenene, Munyaradzi**	Effect of larval density and additional anchoring surface on the life-history traits of a laboratory colonized *Anopheles funestus* strain
		15:20 –15:35 (PhD, 15min)	**Noeth, Kayla**	Hormetic responses of a laboratory strain of *An. arabiensis* to four agricultural pollutants and implications on vector control programs
		15:35-15:40 (MSc, 5x5)	**Chihamba, Faith**	Essential oil constituents as insecticide resistance reversal agents
		15:40-15:45 (MSc, 5x5)	**Grant, Taylya**	The inhibitory effect of copper chelation on *Anopheles* larvae
		15:45 –15:50	**All in session**	Open question session
Concluding remarks	**Dr Ashley Burke**	15:50 – 16:00	**Prof Basil Brooke**	
Awards	**Prof Robyn van Zyl**	16:00 – 16:30	**Prof Lizette Koekemoer**	Lifetime achievement award **Prof Miles Markus**
			Ms Huang-miao Chen	WRIM Logo Hunt Prize
Closure			**Prof Lizette Koekemoer**	Closure of WRIM Postgraduate Research Day 2024
A job well done		16:30 – 17:00	Networking Tea and snacks	

THANK-YOU EVERYONE FOR PARTICPATING IN THE SECOND WRIM RESEARCH DAY

LIFE-TIME ACHEIVEMENT AWARD

Professor Miles Markus has contributed to the field of Malaria with over 160 publications and graduate numerous postgraduate students. His contribution and support to WRIM is recognized at the 2024 WRIM Postgraduate Research Day by being awarded the Biennial Life-Time Achievement Award. Prof Markus has inspired us all with his enthusiasm and unwavering commitment to making a difference in our field, leaving a lasting impression on each of us. Each anecdote and piece of advice he has shared resonates deeply with all of us, sparking valuable conversations. Prof Markus is always willing to share his experiences and challenges and provides us with invaluable insights into the realities of malaria control.

Professor Markus is a graduate of four institutions: Imperial College London; London School of Hygiene & Tropical Medicine; University of Pretoria; and University of the Witwatersrand. He has science, biomedical and language qualifications and is the person who coined the term "hypnozoite" for the stage that has been thought to cause relapse of malarial infections, a phenomenon known since ancient times. Many of his numerous research-related articles, mostly sole-authored, have been published in high-impact journals, including "Nature" and "Science". His most recent award (2023) is the Elsdon-0ew Medal of the Parasitological Society of Southern SAfrica (their highest honour). In terms of student evaluation of lecturing performance, involving both large (e.g. medical students) and small classes, he has been recognized as a distinguished teacher. Professor Markus is based in the School of Animal, Plant and Environmental Sciences, Faculty of Science, University of the Witwatersrand. He is also associated with the Wits Research Institute for Malaria (WRIM) in the Faculty of Health Sciences at the same institution; and is a member of a research laboratory at Ohio State University, USA.

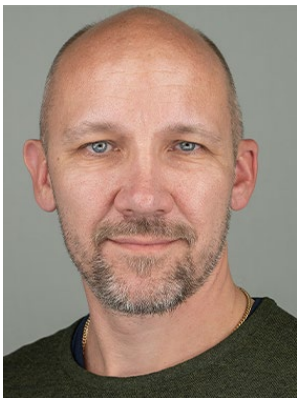
LIFE-TIME ACHEIVEMENT AWARD PRESENTATION – 25TH APRIL 2024

HIDDEN PARASITES IN MALARIA

Following recent research and intellectual analysis, understanding of the life cycle of *Plasmodium* in the human host has changed, as has interpretation in relation to an aspect of the treatment of malaria. These matters will be explained. If you have a plasmodial life cycle diagram amongst your notes from school or university, throw it away. It is out of date.

PLENARY PRESENTATION

24TH APRIL 2024



Controlling mosquito populations genetically

Professor Tony Nolan

Liverpool School of Tropical Medicine

Control of the mosquito vector of malaria has always been our most effective weapon in reducing the burden of this disease. However, alternative control methods are needed. One such method is genetic control – the deliberate release of insects carrying altered genetic traits into a population in order to affect its potential to transmit. Gene drive is a particularly powerful form of genetic control that strongly biases the inheritance of specific genetic traits, allowing these to rapidly spread in a population and, in many examples, reach fixation. This type of technology has transformative potential since it allows the specific targeting of only those mosquito species responsible for malaria transmission and it can spread from a very low initial release ratio, meaning logistical barriers to implementation are much lower.

In this talk Prof Nolan will describe how he has developed gene drives in the laboratory that are designed to spread in a population and cause its suppression by interfering with female fertility, how he tested these and addressed some of the challenges they present. Along the way he developed a range of functional genetics tools that are shedding light on the genetic determinants of what makes the mosquito a good vector and I will illustrate these with examples.

Professor Nolan completed his PhD studies at Imperial College, focusing on "A transformational technology for mosquito vectors of malaria". He then proceeded to do his post-doctoral studies at the University of Rome and the University College of London, looking at genome defense mechanisms against genetic parasites and ageing. Currently, he is the Chair of Functional Genetics at the LSTM.

Professor Nolan's research focuses on three main areas:

1 - Genetic control of mosquito populations in order to control malaria transmission.

2 - Development of functional genetics tools for the study of key mosquito traits (such as insecticide resistance, mosquito fertility, bloodmeal digestion etc.).

3 - Developing and sharing capacity around the molecular biology and genetics - both knowledge and practical infrastructure - that is needed to accompany the implementation and monitoring of vector control programmes.

15 MINUTE ORAL PRESENTATIONS

Design, molecular docking, drug-likeness and ADMET prediction of chalcones as novel *Plasmodium falciparum* cytochrome bc_1 (cyt bc_1) inhibitors

Agarwal P [1,2], van Zyl RL [1,2]

[1] Pharmacology Division, Department of Pharmacy and Pharmacology, Faculty of Health Sciences, University of the Witwatersrand, 7 York Road, Johannesburg 2193, South Africa; [2] WITS Research Institute for Malaria, Faculty of Health Sciences, University of the Witwatersrand, 7 York Road, Johannesburg 2193, South Africa.

A novel series of chalcones were designed by using the rational drug design approach with Schrödinger software suite. Newly designed compounds were screened virtually for antimalarial effectiveness in both, sensitive and resistant models and for drug-likeness and ADMET prediction using *in silico* tools. Compounds were designed using interactive pose prediction and R-Group enumeration, followed by molecular docking that was performed against the X-ray structure PDB ID: 3CX5 representing the cytochrome bc_1 complex enzyme. This study reports antimalarial potential of novel chalcone compounds as drug-like molecules which can be further optimised and developed as potent *Plasmodium* cyt bc_1 inhibitors.

Heat shock factor (HSF) expression and its effect on life table parameters in the main African malaria vector *Anopheles funestus*

Bloch, N. W. [1,2] Koekemoer, L. L. [1,2], Dahan-Moss, Y. L. [1,2]

[1] Wits Research Institute for Malaria, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa; [2] Centre for Emerging Zoonotic & Parasitic Diseases, National Institute for Communicable Diseases, a Division of the National Health Laboratory Service, Johannesburg, South Africa.

Anopheles funestus is a major malaria vector in southern Africa and therefore it is essential to control this species. This necessitates studying the underlying factors that influence the biology of this species. In mosquitoes, the transcription factor, heat shock factor (HSF), is a key component in physiological and behavioural processes. However, there is limited information regarding HSF and its role in *An. funestus*. The aim of this study is to investigate the HSF expression in the different life stages of *An. funestus*, and its biological role on life table parameters in this species. The baseline expression of HSF in all life stages of *An. funestus* were investigated using real-time PCR (RT-qPCR). *Anopheles funestus* adults were fed with sugar water containing an optimized concentration of short interfering RNA that targets the HSF gene (HSF-siRNA). The expression of HSF in HSF-siRNA treated *An. funestus* adults was confirmed via RT-qPCR. The phenotypic effect of HSF-siRNA on longevity and reproduction in *An. funestus* was examined. HSF transcript abundance analysis on the four life stages in *An. funestus*, showed that it was significantly lower at the egg and pupal stages compared to the larval and adult stages. The HSF-siRNA knockdown reduced both longevity and reproduction in *An. funestus*. In conclusion, HSF is expressed at varying levels in all life stages, and might play a role in developmental processes. HSF plays a role in survival and reproduction, indicating the pleiotropic effect of HSF in *An. funestus*.

Biting back: screening the MMV Global Health Priority Box for endectocidal activity against malaria vector mosquitoes

Burke, A.M. [1,2], Aswat, A. [1,2] & Koekemoer, L.L. [1,2]

[1] Wits Research Institute for Malaria, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa. [2] Centre for Emerging Zoonotic and Parasitic Diseases, National Institute for Communicable Diseases of the National Health Laboratory Service, Johannesburg, South Africa.

The Global Health Priority Box grants scientists access to compounds with confirmed pathogen and vector activity. The WRIM entomology group screened the GHP Box for endectocidal activity against malaria vectors. Endectocides, which target both internal and external parasites, present a promising avenue for vector control. Using a high throughput endectocide membrane feeding assay, 240 MMV compounds were evaluated for efficacy against *Anopheles coluzzii* female mosquitoes. While 20 compounds showed significant endectocidal activity (>80% mortality) and 9 exhibited repellence (<20% feeding rate), no novel potent endectocides emerged. This investigation not only underscores the complexity of vector control but also highlights the need for continued innovation and exploration in the field.

The effect of immune stimulation on histone modification of the major malaria vector *Anopheles arabiensis* Patton (Diptera: Culicidae)

Chen C-Y [1,2], Munhenga G [2], Oliver SV [2,1]

[1] Wits Research Institute for Malaria, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa; [2] Centre for Emerging Zoonotic and Parasitic Diseases, National Institute for Communicable Diseases of the National Health Laboratory Service, Johannesburg, South Africa.

The role of epigenetics in the immune response of mosquitoes is unclear. However, what is known is that histone modifications, rather than nucleic acid methylation, underlie epigenetic regulation in insects. Thus, the study aimed to investigate modifications of H3 and H4 histones after immune stimulation of F1 generation of wild *Anopheles arabiensis* sampled from KwaZulu-Natal. Significant differences in histone modifications were found at several H3 and H4 modification sites. The study also revealed several key H3 and H4 modification sites that can be researched further to understand the epigenetics of *An. arabiensis*.

Mating in captivity: An observational study of copulation events between *Anopheles arabiensis* and *An. funestus*

Hape E [1,2], Nkya J [1], Ngonyani A [1], Mabula D [1], Koekemoer L [2] and Okumu F [1,2,3]

[1] Ifakara Health Institute, Morogoro, United Republic of Tanzania, [2] University of the Witwatersrand, Johannesburg, South Africa, [3] University of Glasgow, Glasgow, United Kingdom.

Understanding the reproductive biology of malaria vectors, especially *Anopheles arabiensis* and *An. funestus*, offers crucial insights into population dynamics. Courtship behaviours and mating rituals influence mating success, affecting generation contributions. The study examined copulation dynamics between these species, including FUMOZ and FUTAZ strains. It comprised 24-hour observational periods over 16 consecutive days, with three replicates per species housing around 1500 mosquitoes each. Results show distinct temporal patterns in copulation behaviour: *An. arabiensis* initiates mating at two days, peaking at three; *An. funestus* (FUTAZ) starts at three days, peaking at seven, while FUMOZ begins at four days, peaking at ten. Dissection revealed successful reproductive outcomes, with 98% showing sperm transfer and mating plug delivery. Understanding species-specific mating dynamics is vital for targeted vector control strategies to disrupt their reproductive cycle and mitigate malaria transmission.

Exploring the potential of using the sugar water diet to enhance *An. funestus* laboratory colonisation

Kalonji MN [1], Dahan-Moss YL [2], Koekemoer LL [3]

[1] Wits Research Institute for Malaria, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa; [2] Centre for Emerging Zoonotic & Parasitic Diseases, National Institute for Communicable Diseases, a Division of the National Health Laboratory Service, Johannesburg, South Africa.

Anopheles funestus is a major malaria vector in Africa. As novel control methods for this vector are required, data is needed regarding sugar diet effects on the species' biology to optimise mass-rearing protocols. Sugar diet impacts on key lifetable parameters in *An. funestus* were assessed. Mosquitoes were fed on different sugar water concentrations for various experiments. Higher sugar water concentration diets increased mosquito mating success, fertility, flight performance, and survival rates. This study definitively shows the importance of sugar in *An. funestus* and how this can enhance colonisation; especially when additional vector control methods rely on using laboratory-reared mosquitoes.

A fight for dominance: *Microsporidia MB* abundance and intensity in germline and somatic tissues

Makhulu E [1], Onchuru T [1], Wairimu A [1], Muthoni [1], Gichuhi [1], Otieno F [1], Koekemoer L [1], Herren J [8]

[1] Wits Research Institute for Malaria, Faculty of Health Sciences, University of the Witwatersrand.

Because *Microsporidia MB* interferes with *Plasmodium* development and is predominately vertically transmitted, it can be utilized as a symbiont-based malaria control strategy. However, the rate of transmission is determined by symbiont intensity which may affect its fixation in the population. Factors like host age, nutritional changes, and nutritional reserves that affect its proliferation and abundance, affect its intensity and eventual transmission. This study, therefore, investigated tissue localisation and tropism dynamics of *Microsporidia MB* in relation to age, and nutritional changes in adult *Anopheles arabiensis*. Also, its impact on host nutrition reserves and host fitness during nutritional deprivation was investigated

Variable effects of urban and rural pollutants on the life history of the major malaria vector *Anopheles arabiensis* (Diptera: Culicidae),

Makumbe HH[1,2]; Rasikhanya W[1,2]; Chen CY[1,2] and Oliver SV [1,2]

[1] Centre for Emerging Zoonotic & Parasitic Diseases, National Institute of Communicable Diseases, [2] Wits Research Institute for Malaria, Faculty of Health Sciences, University of the Witwatersrand.

Anopheles arabiensis is rapidly adapting to breeding in polluted water. This has resulted in this vector expanding its traditional niche of breeding associated with agriculture to adapting to urban pollutants. However, it is unclear as to whether the species copes better with pollutants associated with urban or rural pollutants. Nitrogenous pollutants were used as a proxy for rural pollutants and sulphur-based pollutants a proxy for urban pollutants. The effects of larval exposure in adults were affected by insecticide resistant phenotype. Nitrogenous pollutants had a more positive effect than sulphur-based pollutants. This has implications for adaption in this species.

The impact of climatic factors on temporal mosquito distribution and population dynamics in an area targeted for Sterile Insect Technique pilot trials.

Mazarire T[1,2], Lobb l[1,2], Newete S[1,2], and Munhenga G[1,2]

[1] Centre for Emerging Zoonotic & Parasitic Diseases, National Institute of Communicable Diseases, [2] Wits Research Institute for Malaria, Faculty of Health Sciences, University of the Witwatersrand.

The persistence in malaria prevalence globally is partly attributed to climatic factors due to mosquitoes' sensitivity to environmental conditions. It is widely accepted that climate affects the mosquito life history traits, however, its precise role in determining mosquito distribution and population dynamics is not fully understood. This study aimed to investigate the influence of various climatic factors on the temporal distribution of *An. arabiensis* populations in Mamfene, South Africa between 2014 to 2019. Time series analysis, wavelet analysis, cross-correlation analysis, and regression model combined with the autoregressive integrated moving average (ARIMA) model was utilised to assess the relationship between climatic factors and *An. arabiensis* population density. In total 3 826 adult *An. arabiensis* collected was used for the analysis. ARIMA (0,1,2) (0,0,1)12 models closely described the trends observed in *An. arabiensis* population density and distribution. The wavelet coherence and time-lagged correlation analysis showed positive correlations between *An. arabiensis* population density and temperature (r = 0.537), humidity (r = 0.495) and rainfall (r = 0.298) whilst wind showed negative correlations (r = -0.466). Regression model showed that rainfall (p = 0.0029), temperature (p = 0.254), and humidity (p = 0.0103) as significant predictors for forecasting *An. arabiensis* abundance. The extended ARIMA model (AIC = 102.08) was a better fit for predicting *An. arabiensis* abundance compared to the basic model. *An. arabiensis* still remains the predominant malaria vector in the study area with population varying seasonally. Climate variables were found to have varying effects on the distribution and abundance of *An. arabiensis*. Timely mosquito population and climate information enhance effective targeted vector control in SIT programs and reduction in malaria risk.

Impact of insecticide exposure on *Plasmodium falciparum* development in resistant *An. gambiae* mosquitoes

Adandé Medjigbodo [1], Laurette Djossou [1], Oswald Y. Djihinto [1], David Weetman [2], Martin J. Donnelly [2], and Luc S. Djogbénou [1,2]

[1] Tropical Infectious Diseases Research Centre (TIDRC), University of Abomey-Calavi, 01BP 526, Cotonou, Benin. [2] Department of Vector Biology, Liverpool School of Tropical Medicine, Pembroke Place, Liverpool L3 5QA, United Kingdom.

The aim of current study was to evaluate whether the exposure to permethrin and bendiocarb affects the development of *P. falciparum* in different strains of *An. gambiae*. Mosquito females were pre-exposed to 0.75% permethrin and 0.1% bendiocarb insecticides. Exposed mosquitoes were then blood fed on a *P. falciparum* gametocyte culture. In both used laboratory and near-field mosquitoes, permethrin and bendiocarb impaired *P. falciparum* within-vector development. We showed that, sublethal doses of permethrin and bendiocarb, can interfere with *P. falciparum* development inside *An. Gambiae*. In-depth understanding of this mechanism could help to predict the real impacts of insecticide applications on malaria transmission.

The detection and effect of microplastics on the gut microbiota of the major malaria vector, *Anopheles arabiensis* Patton (Diptera: Culicidae)

Misser S [1,2], Chen C [1,2], Oliver S.V [2,1]

[1] WITS Research Institute for Malaria, University of the Witwatersrand; [2] Vector Control Reference Laboratory, National Institute for Communicable Diseases.

The microbiota of the midgut of mosquitoes is important for mosquito life history. The larval environment influences gut microbial composition. In this study, we determined the fate of microplastic and the effect of microplastic and plastic additives on the gut microbiota of adult females of two laboratory strains of *An. arabiensis*. An insecticide-susceptible and insecticide-resistant strain were used. Latex beads were observed in the larval midgut and adult testes. 16S rRNA gene sequencing was performed on the midgut of plastic-treated and untreated three-day-old adult females. Larval exposure to plastic alters the microbiota, particularly in the absence of a resistance phenotype.

Hormetic responses of a laboratory strain of *An. arabiensis* to four agricultural pollutants and implications on vector control programs

Noeth KP [1,2], Oliver SV [1,2], Munhenga G [1,2]

[1] Centre for Emerging Zoonotic & Parasitic Diseases, National Institute of Communicable Diseases, [2] Wits Research Institute for Malaria, Faculty of Health Sciences, University of the Witwatersrand.

Hormesis is a physiological phenomenon where exposure to a toxicant or other stressor at low doses results in a stimulatory effect of one or more life history traits, while at high doses an inhibitory effect is observed. Hormetic effects was investigated for a laboratory stain of *An. arabiensis* by setting up a pollution gradient for four different agricultural pollutants. Preliminary results indicate that sublethal doses of glyphosate may elicit a hormetic response and that tolerance to styrene comes at a fitness cost. The implications of the results on vector control programs are discussed.

The dynamic gut microbiota of laboratory reared, the first filial population, and wild population of the major malaria vector *Anopheles arabiensis* Patton (Diptera: Culicidae)

Singh A [1,2], Allam M [3], Chan WYA [4,5], Ismail A [4], Oliver SV [1,2]

[1] Centre for Emerging Zoonotic and Parasitic Diseases, NICD. [2] Wits Research Institute for Malaria, School of Pathology. [3] Department of Genetics and Genomics, College of Medicine and Health Sciences, United Arab Emirates University. [4] Sequencing Core Facility, NICD. 5: Department of Veterinary Tropical Diseases, Faculty of Veterinary Science, University of Pretoria.

The gut microbiota of mosquitoes is a potential target for vector control interventions. This study characterised the gut microbiota of laboratory reared strains, the F1 population and the wild population of three-day old *An. arabiensis* females. The F1 population and wild population had microbial diversity than laboratory reared strains. Several *Plasmodium* protective genera were identified in the F1 population and wild populations. These genera can potentially be used for vector control as these are native symbionts. In conclusion, these findings have implications for using gut bacteria as a vector control intervention in South Africa.

Effect of larval density and additional anchoring surface on the life-history traits of a laboratory colonized *Anopheles funestus* strain

Zengenene MP [1,2], Munhenga G [1,2], Okumu F [3], Koekemoer LL [1,2]

[1] Wits Research Institute for Malaria, School of Pathology, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa. [2] Vector Control Reference Laboratory, Centre for Emerging Zoonotic and Parasitic Diseases, National Institute for Communicable Diseases, National Health Laboratory Services, Johannesburg, South Africa. [3] Department of Environmental Health and Ecological Sciences, Ifakara Health Institute, Ifakara, Tanzania.

Optimal rearing conditions, inclusive of larval rearing density, are critical for sustained mosquito productivity. There is limited information on favourable conditions for the larval rearing of *Anopheles funestus*. This work investigated the effects of larval rearing densities and additional anchoring surface on *An. funestus* development using a life table approach. Rearing larvae at high densities extended the larval developmental time and reduced adult productivity. Adding an extra larval anchoring surface when rearing larvae at high density resulted in extended larval developmental time, increased larval survivorship and produced bigger adults. These findings improve our understanding of the relationship between larval density and developmental traits.

5X5 ORAL PRESENTATIONS

Essential oil constituents as insecticide resistance reversal agents

Chihamba TF [1,2], Rants'o T [1,2], Koekemoer L [2], van Zyl RL [1,2]

[1] Pharmacology Division, Department of Pharmacy and Pharmacology, Faculty of Health Sciences, University of the Witwatersrand, 7 York Road, Johannesburg 2193, South Africa ; [2] WITS Research Institute for Malaria, Faculty of Health Sciences, University of the Witwatersrand, 7 York Road, Johannesburg 2193, South Africa.

Reducing malaria prevalence with insecticides has been effective, but combating insecticide resistance in vectors necessitates the development of new insecticides or resistance reversal agents. Essential oil constituents have proven to effectively kill the malaria vector. Bisabolol, cis-, trans-nerolidol, cis-nerolidol, linalool and linalyl acetate activity was determined against MBN, FANG and FUMOZ-R *Anopheles* species. The essential oil constituents proved to be highly effective at concentrations from 1% with 100% mortality. Linalyl acetate proved to be the most active, knocking down mosquitoes after 5min, followed by cis-nerolidol in 10min. These essential oil constituents are effective insecticides against sensitive and resistant *Anopheles* species

HPLC method development and validation of (-)-alpha-Pinene

Chiliza U [1,2], Koekemoer L [1,2], van Eyk AD [1,2], van Zyl RL [1,2]

[1] Pharmacology Division, Department of Pharmacy and Pharmacology, Faculty of Health Sciences, University of the Witwatersrand, 7 York Road, Johannesburg 2193, South Africa ; [2] WITS Research Institute for Malaria, Faculty of Health Sciences, University of the Witwatersrand, 7 York Road, Johannesburg 2193, South Africa.

Alpha-pinene, a terpene found in plant essential oils possesses mosquito repellent properties comparable to DEET. To find novel mosquitocidal and repellent compounds, a sensitive analytical HPLC method was developed and verified to accurately measure (-)-α-pinene. The method used isocratic mobile phase conditions and was validated according to ICH guidelines. Three concentrations were selected for robustness, accuracy, and precision. The calibration curves were linear, with LOD and LOQ values of 198.25 (v/v) and 600.75 (v/v), respectively. The method is simple, specific, precise, accurate, and robust, making it suitable for routine analysis of (-)-α-pinene.

The inference of OTC phytomedicines on oxidative stress, metal homeostasis, and *Plasmodium falciparum* survival

Govender V [1,2], van Eyk A [1,2], van Zyl RL [1,2]

[1] Pharmacology Division, Department of Pharmacy and Pharmacology, Faculty of Health Sciences, University of the Witwatersrand, 7 York Road, Johannesburg 2193, South Africa ; [2] WITS Research Institute for Malaria, Faculty of Health Sciences, University of the Witwatersrand, 7 York Road, Johannesburg 2193, South Africa.

Drug resistant malaria is a major contributor to its persistence in Africa. Phytomedicines are promising candidates in the search for novel treatments. Do extracts of *Camelia sinensis*, *Viscum album*, *Siphonochilus aethiopicus*, and *Warburgia salutaris* alter oxidative stress, metal homeostasis, and *P. falciparum* survival? DPPH, ferrous ion chelation, and pLDH assays were undertaken. At 1 mg/mL, all extracts, save the aqueous extract of *S. aethiopicus*, had DPPH and ferrous chelating activity (>95%); the organic extracts of *V. album*, *S. aethiopicus*, *W. salutaris*-(A), and *W. salutaris*-(B) possessed antiplasmodial activity (>60%). Phytomedicines can alter oxidative stress, metal homeostasis, and *P. falciparum* survival.

The inhibitory effect of copper chelation on *Anopheles* larvae

Grant T [1,2], Mahlangu J [1,2], Van Zyl RL [1,2]

[1] Pharmacology Division, Department of Pharmacy and Pharmacology, Faculty of Health Sciences, University of the Witwatersrand, 7 York Road, Johannesburg 2193, South Africa ; [2] WITS Research Institute for Malaria, Faculty of Health Sciences, University of the Witwatersrand, 7 York Road, Johannesburg 2193, South Africa.

Exploring the role of copper and copper chelators in mosquito larvae control presents a novel avenue for vector control strategies. Copper ions play crucial roles in essential metabolic pathways in mosquitoes, and targeting these pathways could offer effective control measures. The study conducted investigated the effects of neocuproine and its copper (II) chelate on mosquito larvae, demonstrating a higher percentage of larval lethality compared to individual compounds. Combining the chelate with propoxur enhances insecticide efficacy at lower concentrations. These findings indicate promising potential for neocuproine and its chelate in vector control. Further research is needed to understand their mechanisms and long-term efficacy.

Unlocking history: Assessing DNA extraction kits for ancient *Anopheles* specimens in the museums

Jamesboy E [1,2], Snyman L [3], Coetzee M [1.2], Koekemoer L [1,2] and Dahan-Moss Y[1,2]

[1] Wits Research Institute for Malaria, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa; [2] Centre for Emerging Zoonotic & Parasitic Diseases, National Institute for Communicable Diseases, a Division of the National Health Laboratory Service, Johannesburg, South Africa. [3] Invertebrate Zoology, Royal Alberta Museum, Canada.

Accurate species identification within the Anophelinae species complex is vital for effective malaria control. Amplification of COI gene aids species identification and this relies on precise reference material, such as type museum specimens. However, DNA extraction and amplification from museum specimens is challenging. In this study five commercial kits were used to extract DNA from single-leg museum specimens, which was followed by PCR amplification. From the five kits, DNeasy blood and tissue kit (Qiagen) performed best for the extraction of DNA followed by successful amplification of COI. This kit is recommended for DNA extraction from small tissue museum specimens.

Assessing potential bottlenecks in the mass production of sterilised male Anopheles arabiensis for a South African SIT feasibility study

Machweu MT [1,2], Mashatola T [2], Munhenga G [2] and Samuel M [2,3]

[1] School of APES; [2] Centre for Emerging Zoonotic & Parasitic Diseases, National Institute for Communicable Diseases, a Division of the National Health Laboratory Service, Johannesburg, South Africa; [3] International Atomic Energy Agency (IAEA); [4] Wits Research Institute for Malaria, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa

The feasibility of a sterile insect technique (SIT) targeting the South African malaria vector Anopheles arabiensis is currently being investigated. Mass production of the mosquito is central to a functional SIT program and understanding the capacity and limitations are vital in meeting release requirements. Over the course of the production pipeline, mosquitoes perish at identifiable bottlenecks primarily due to rearing and handling methods. Quantification of these losses is important in order to estimate production capacity and to assess quality control. In this study, we will investigate the loss of mosquitoes at various bottlenecks in the production of sterile male mosquitoes.

The properties of 4-allyl-2-methoxyphenol derivatives as possible larvicides and metal chelators

Malan S [1,2], Mahlangu J [1,2], Rants'o TA [1,2], Van Zyl RL [1,2]

[1] Pharmacology Division, Department of Pharmacy and Pharmacology, Faculty of Health Sciences, University of the Witwatersrand, 7 York Road, Johannesburg 2193, South Africa ; [2] WITS Research Institute for Malaria, Faculty of Health Sciences, University of the Witwatersrand, 7 York Road, Johannesburg 2193, South Africa.

Novel agents to combat the *Anopheles* mosquito are required as the development of resistance to synthetic insecticides is on the rise. The study investigated the insecticidal and metal chelating properties of derivatives of 4-allyl-2-methoxyphenol, a phenol known for insecticidal activity. 2-Methoxy-4-propenylphenol showed strong ferrous ion chelation but limited copper chelation. 1,2-Dimethoxy-4-propenylbenzene demonstrated potent larvicidal effects against *Anopheles* larvae, outperforming propoxur with low toxicity to *Artemia*. The study also employed *in silico* target predictions to identify potential targets of the derivatives. These findings highlight the potential of novel bio-agents derived from essential oils in combating malaria transmission by targeting *Anopheles* mosquitoes.

Analysis of a 3D-printed irradiation canister for bulk *Anopheles arabiensis* sterilization in a South African sterile insect technique

Samuel M [1,2,3,4], Mashatola T [1,2,3,4], Beukes P [4], and Munhenga G [1,2,3,4]

[1] Wits Research Institute for Malaria, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa; [2] Centre for Emerging Zoonotic & Parasitic Diseases, National Institute for Communicable Diseases, a Division of the National Health Laboratory Service, Johannesburg, South Africa; [2] International Atomic Energy Agency (IAEA); [4] iThemba Labs.

The sterile insect technique (SIT) is a biologically friendly pest management approach that uses the mass production and release of sterilized insects to inundate and ultimately suppress wild target populations. SIT is currently being considered as a supplementary vector control approach in South Africa, where residual malaria persists as a result of the primary mosquito vector *Anopheles arabiensis*. Sterility is induced in the male insects using ionizing radiation and an X-Ray irradiation protocol is currently being optimized. In this study, we will assess the applicability of a 3D-printed irradiation canister for bulk sterilization of *An. arabiensis*.

2X2 ORAL PRESENTATIONS

Knowledge, attitudes and practices of malaria management in the Ehlanzeni District, Mpumalanga, South Africa

Dennis L [1,2], Schmollgruber S [1,3], Van Zyl RL [1,2]

[1] Pharmacology Division, Department of Pharmacy and Pharmacology, Faculty of Health Sciences, University of the Witwatersrand, 7 York Road, Johannesburg 2193, South Africa ; [2] WITS Research Institute for Malaria, Faculty of Health Sciences, University of the Witwatersrand, 7 York Road, Johannesburg 2193, South Africa.

With malaria remaining a relevant public health concern in the region, understanding community perceptions and behaviours towards prevention and treatment is critical for effective control efforts. A questionnaire focused on a malaria-endemic population will undergo a Delphi review. Thereafter the self-administered questionnaires will be used to assess the knowledge, attitudes, and practices among residents in the Ehlanzeni district of South Africa. Findings will be used to inform tailored interventions to help enhance community engagement, improve adherence to preventive measures, and optimize case management strategies. Ultimately, this research aims to contribute to the ongoing efforts towards malaria elimination in South Africa.

The repellent effects of naturally derived essential oils against the anopheline mosquitoes

Rakgoroana MP [1,2], Mahlangu J [1,2], Koekemoer L [1,2], Van Eyk AD [1,2], Van Zyl RL [1,2]

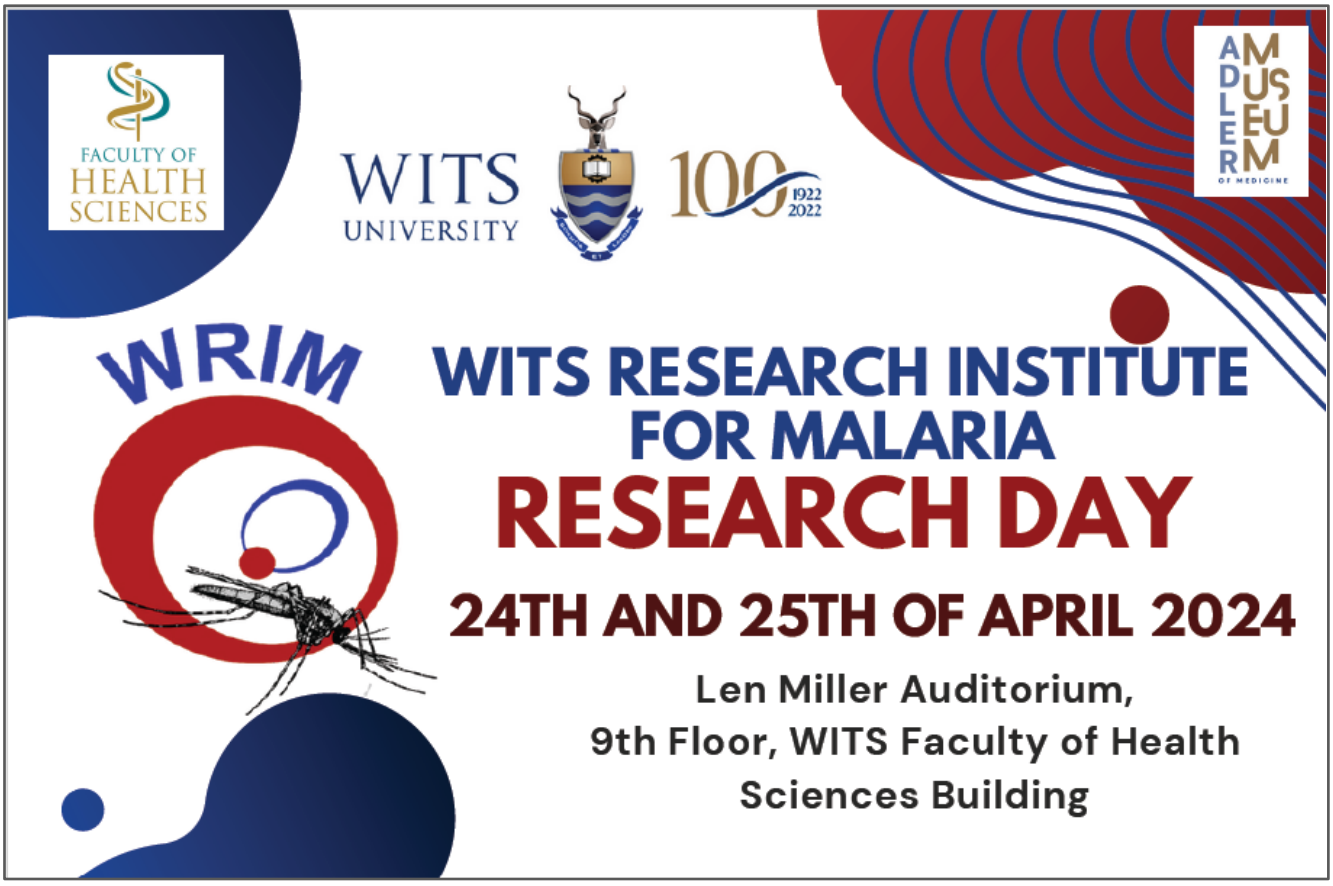
[1] Pharmacology Division, Department of Pharmacy and Pharmacology, Faculty of Health Sciences, University of the Witwatersrand, 7 York Road, Johannesburg 2193, South Africa ; [2] WITS Research Institute for Malaria, Faculty of Health Sciences, University of the Witwatersrand, 7 York Road, Johannesburg 2193, South Africa

Repellents are a proven chemo-preventative method used to reduce malaria transmission and malaria associated deaths. Commercial repellents as well as natural essential oils (EOs), such as citronella are commonly used. This study will investigate additional EOs, as well as further optimize their efficacy by assessing the EO constituents alone and in combination against anopheline mosquitoes. To achieve this GC-MS will determine the chemical profile of the EOs, before the repellency is assessed on human volunteers. The ability of the EOs and EO constituents to permeate the dermal layer will be assessed and quantitated using HPLC analysis.

LIST OF ATTENDEES AND PRESENTERS

Agarwal, Priyanka	2286575@students.wits.ac.za
Aswat, Ayesha	ayesha.aswat@wits.ac.za
Bloch, Nerissa	nerissa.wendy@gmail.com
Boanyah, Godfred Yaw	gboanyah@icipe.org
Brooke, Basil	basilb@nicd.ac.za
Burke, Ashley	ashley.burke@wits.ac.za
Chen, Chia-Yu	chia-yu.chen@wits.ac.za
Chen, Huang-miao	1656984@students.wits.ac.za
Chihamba, Faith	879896@students.wits.ac.za
Chiliza, Unathi	1657318@students.wits.ac.za
Dahan-Moss, Yael	yaelda@nicd.ac.za
Dennis, Lauren	laurendennis1@students.wits.ac.za
Djihinto, Oswald	oswalddjihinto@outlook.fr
Erlank, Erica	erica.erlank@wits.ac.za
Frean, John	johnf@nicd.ac.za
Govender , Venisha	1645329@students.wits.ac.za
Grant, Taylya	2345360@student.wits.ac.za
Hape, Emmanuel Elirehema	ehape@ihi.or.tz
Jamesboy, Eunice	eunicej@nicd.ac.za
Kaiser, Maria	Mariak@nicd.ac.za
Kalonji, Mbuyi	1043541@students.wits.ac.za
Koekemoer, Lizette	lizette.koekemoer@wits.ac.za
Korsah, Emmanuel Kwame	Emmanuel.korsah1@wits.ac.za
Lala, Sahil	Sahil.Lala@wits.ac.za
Langa, Zandile	zandilel@nicd.ac.za
Lazarus, Faith	Faith.Lazarus@wits.ac.za
Machweu, Thato	2436040@students.wits.ac.za
Mahlangu , Jabu	Jabu.mahlangu@wits.ac.za
Mahlangu , Thando	2334037@students.wits.ac.za
Makhulu, Edward	emakhulu@icipe.org

Makumbe, Hattie	hattie.makumbe@wits.ac.za
Malan, Sune	1849730@students.wits.ac.za
Markus, Miles	miles.markus@wits.ac.za
Mashatola, Thabo	thabomas@nicd.ac.za
Matamba, Avhatakali	avhatakalim@nicd.ac.za
Mazarire, Theresa	1316540@students.wits.ac.za
Medjigbodo, Adandé A.	medjis2010@yahoo.fr
Misser, Shristi	1388499@students.wits.ac.za
Mpukwana, Pumela	Pumelam@nicd.ac.za
Noeth, Kayla	kayla.noeth@gmail.com
Nolan, Tony	Tony.nolan@lstmed.ac.uk
Oliver, Shune	shuneo@nicd.ac.za
Rakgoroana, Mogale Patrick	2185274@students.wits.ac.za
Ramatshimbila, Mulanga	treaty2003@gmail.com
Sabjee, Safeera	safeerasabjee128@gmail.com
Samuel, Michael	michaels@nicd.ac.za
Schmollgruber, Shelley	shelley.schmollgruber@wits.ac.za
Sebuseng , Letlhogonolo	reamogetsesebuseng@gmail.com
Sekgele, Windy	windy@nicdac.za
Seroke, Penelope	ntshabeleng.penelope@gmail.com
Singh, Ashmika	ashmikasingh1@gmail.com
Sriruttan , Charlotte	CharlotteS@nicd.ac.za
Touffie, Zia	2427817@students.wits.ac.za
Tshabalala, Thandokuhle	thandokuhlet@nicd.ac.za
van Eyk, Armorel	Armorel.vaneyk@wits.ac.za
Van Zyl, Robyn	Robyn.Vanzyl@wits.ac.za
Venter, Nelius	nelius.venter@wits.ac.za
Zengenene, Munyaradzi Prince	1932617@students.wits.ac.za



GOOD LUCK WITH YOUR RESEARCH AND STUDIES

NEXT WRIM POSTGRADUATE RESEARCH DAY – APRIL 2026