Indian Journal of Animal Sciences **94** (4): 362–368, April 2024/Article
https://doi.org/10.56093/ijans.v94i4.146821

Impact of varying doses of Moringa leaf extract supplementation in the cryopreservation media on sperm quality, antioxidant capacity and antimicrobial activity of frozen-thawed buck spermatozoa

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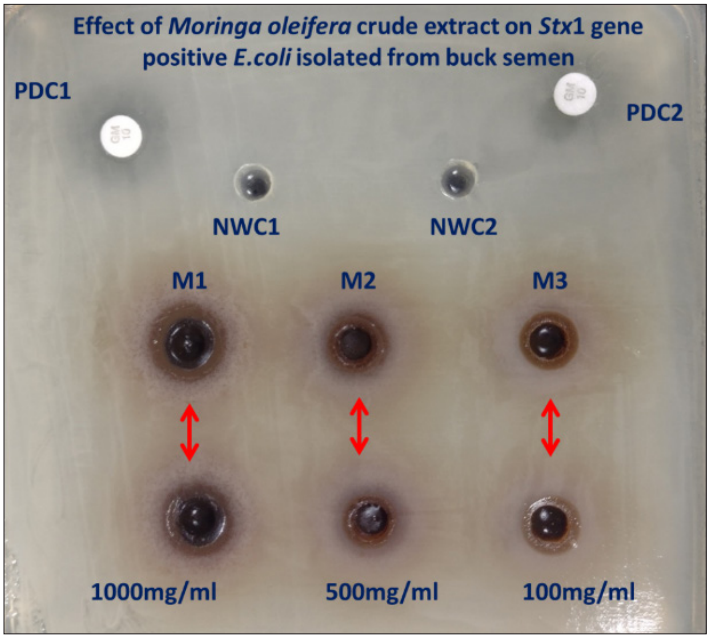
Received: 27 December 2023; Accepted: 23 February 2024

Supplementary Fig. 1. A. Gram's stained microscopic smear of *E. coli* appearing gram negative (pink) cocco-bacilli under 100X oil immersion objective; B. MacConkey's agar showing round raised pinkish lactose fermenting colonies of *E. coli*; C. Characteristic green metallic sheen on EMB agar.



Supplementary Fig. 2. Effect of *Moringa oleifera* leaf aqueous extract on *E. coli*. PDC1 and 2 – positive disk control – Gentamicin (10 µg, CLSI) – 14 mm; NWC1 and 2 – Negative well control with only diluent to check any non-specific Zone of Inhibition, ZoI @ 0 mm; M1 – 1000 mg/ml of *Moringa* extract used @10 µl/well, ZoI @13.0±0.70 mm; M2–500 mg/ml of *Moringa* extract used @10 µl/well, ZoI @10.25±0.62 mm; M3–100 mg/ml of *Moringa* extract used @10 µl/well, ZoI @6.75±1.10 mm.

Supplementary Table 1. Oligonucleotides details used for multiplex cPCR for confirmation of *E. coli* isolates from semen

Name of Oligonucleotide	Oligo sequence 5′→3′	Conc. used in PCR master mix	Amplicon size
LacY-F	CTACCGGTGAACAGGGTA CG	0.15µM	289 bp
LacY-R	GTCGCTGAAAAACGCACT TC		
LacZ-F	ATGAAAGCTGGCTACAGG AAGG	0.5 µM	517 bp
LacZ-R	CTCCACACAGTTTCGGGTTTTC		
cydA-F	CCGTATCATGGTGGCGTGTGG	0.4 µM	398 bp
cydA-R	GCCGGCTGAGTAGTCGTGGAAG		
uidA-F	CGCCGATGCAGATATTCG	0.4 µM	603 bp
uidA-R	GCTGTGACGCACAGTTCATAG		
phoA-F	GGTAACGTTTCACCGCAGAGTTG	0.6 µM	468 bp
phoA-R	CAGGGTTGGTACACTGTCATTACG		

Supplementary Table 2. Details of primers used for the screening of different genes of *E. coli*

Pathotype	Gene	Primers	Amplicon size (bp)	Primer (pmol)	Publication
ETEC	*It*	F: 5' GGCGACAGATTATACCGTGC 3' R: 5' CGGTCTCTATATTCCCTGTT 3'	450	5.0	(Stacy-Phipps *et al*. 1995)
ETEC	*St*	F: 5' ATTTTTCTTTCTGTATTGTCTT 3' R: 5' CACCCGGTACAAGCAGGATT 3'	190	6.47	(Stacy-Phipps *et al*. 1995)
EPEC	*Bfp* 'A'	F: 5'AATGGTGCTTGCGCTTGCTGC 3' R: 5'GCCGCTTTATCCAACCTGGTA 3'	324	2.5	(Gunzburg *et al*. 1995)
EPEC	*eae*	F: 5' GACCCGGCACAAGCATAAGC3' R: 5' CCACCTGCAGCAACAAGAGG 3'	384	3.88	(Paton and Paton 1998)
STEC	*Stx*1	F: 5' CTGGATTTAATGTCGCATAGTG 3' R: 5' AGAACGCCCACTGAGATCATC 3'	150	3.88	(Paton and Paton 1998)
STEC	*Stx*2	F: 5' GGCACTGTCTGAAACTGCTCC 3' R: 5' TCGCCAGTTATCTGACATTCTG 3'	255	2.5	(Paton and Paton 1998)
EIEC	*ial*	F: 5' GGTATGATGATGATGATGCCA 3' R: 5' GGAGGCCAACAATTATTTCC 3'	650	10.25	(Lopez-saucedo 2003)