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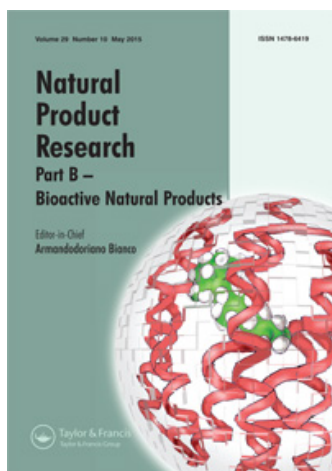
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## SHORT COMMUNICATION

### Natural control of bacteria affecting meat quality by a neem (*Azadirachta indica* A. Juss) cake extract

P. Del Serrone<sup>a\*</sup>, S. Failla<sup>a</sup> and M. Nicoletti<sup>b</sup>

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The antibacterial activity of an ethylacetate neem cake extract (NCE) against bacteria that affect meat quality, namely *Campylobacter jejuni*, *Carnobacterium* spp., *Lactobacillus curvatus*, *Lactobacillus sakei* and *Leuconostoc* sp., is reported. The antibacterial activity was detected using standardised disc diffusion and macrodilution methods. The bacterial growth inhibition zone ranged from  $11.33 \pm 0.58$  to  $22.67 \pm 0.58$  mm (100  $\mu$ L NCE). There is significant difference between the growth inhibition zone of NCE and the control (ciprofloxacin 100  $\mu$ g). The percent of bacterial growth reduction range was  $79.75 \pm 1.53$  to  $90.73 \pm 1.53$  (100  $\mu$ g NCE) as compared with control (without NCE). NCE in different amounts counteracted the growth of all tested bacteria.

**Keywords:** *Azadirachta indica*; neem; neem cake; meat quality; bacterial control

#### 1. Introduction

Meat contamination by pathogenic bacteria has great health consequence and high impact on consumers. The CRA PCM, in collaboration with the Department of Environmental Biology, University Sapienza of Rome, is involved in a project on screening and selection of several plant extracts for their antimicrobial potential use in fresh meat preservation, looking mainly for fixed oils. Neem (*Azadirachta indica* A. Juss) is a monumental tree of Meliaceae family coming from the Indian subcontinent and actually present worldwide. Actually, the importance of neem and its distribution are increasing all over the world due to its beneficial properties, as reported by WHO/UNEP1989 (Nicoletti & Murugan 2013). Here, the preliminary results concerning the use of a neem cake AcOEt extract (NCE) against pathogenic bacteria are reported, in accordance with previous reports on antibacterial activity of neem (Biswas et al. 2002). Neem cake is the waste product of the extraction of the seed oil of neem (*A. indica* A. Juss). Neem cake is potentially useful, involves low cost, sustainable, ecologically friendly raw material and used as fertiliser (Nicoletti, Mariani, et al. 2012; Nicoletti, Maccioni, et al. 2012).

#### 2. Results and discussion

NCE was able to control the growth of all bacteria considered in the experiment. The growth inhibition zone (mm) was in the range of  $11.33 \pm 0.58$  to  $22.67 \pm 0.58$  (Table 1). *Escherichia coli* was the less susceptible and *Leuconostoc* spp. was the most susceptible among tested bacteria.

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Table 1. Antibacterial activity of NCE against spoilage bacteria revealed as growth inhibition zone (mm).

Bacteria	Growth inhibition zone (mm)			
	Treatment			
	NCE (100 µL)	TWN (100 µL)	CFX (100 µL)	WTR (100 µL)
<i>E. coli</i> ATCC® 51813 <sup>TM</sup>	11.33 ± 0.58b	–	28.00 ± 1.00a	–
<i>Brochothrix thermosphacta</i>	18.53 ± 1.15b	–	29.47 ± 1.00a	–
<i>E. faecalis</i> ATCC® 7088 <sup>TM</sup>	16.83 ± 1.18b	–	27.71 ± 1.00a	–
<i>Carnobacterium</i> spp.	10.00 ± 1.00b	–	30.33 ± 1.73a	–
<i>Lactobacillus curvatus</i>	16.33 ± 0.58b	–	23.41 ± 1.00a	–
<i>Lactobacillus sakei</i>	17.50 ± 0.50b	–	27.33 ± 2.08a	–
<i>Leuconostoc</i> spp.	22.67 ± 0.58c	–	32.67 ± 2.89a	–

Notes: Diameter of growth inhibition zones including diameter of disc (6 mm). NCE, neem cake extract; TWN, Tween® 80; CFX, ciprofloxacin (1 mg/mL); WTR, water. Values are given as mean ± SD. Values in a row followed by different lowercase letters are significantly different at  $p \leq 0.05$ .

Table 2. Percent (%) of bacterial GR at different NCE concentrations at 24 h in liquid medium (5 mL final volume) using as reference the control treatment (without NCE).

Bacteria	GR (%)			
	Treatment			
	NCE (100 µg)	NCE (10 µg)	NCE (1 µg)	NCE (0.1 µg)
<i>E. coli</i> ATCC® 51813 <sup>TM</sup>	79.75 ± 1.53d	30.61 ± 1.00bc	27.67 ± 1.33b	30.81 ± 2.08a
<i>B. thermosphacta</i>	80.61 ± 1.15d	59.70 ± 1.00bc	37.58 ± 1.33ab	34.86 ± 1.00a
<i>E. faecalis</i> ATCC® 7088 <sup>TM</sup>	90.61 ± 1.15d	69.70 ± 1.00bc	67.58 ± 1.33ab	64.86 ± 1.00a
<i>Carnobacterium</i> spp.	88.90 ± 1.00d	68.79 ± 1.00abc	69.60 ± 0.00ab	66.68 ± 1.20a
<i>L. curvatus</i>	90.73 ± 1.53d	69.70 ± 2.08bc	68.69 ± 2.00b	62.83 ± 1.73a
<i>L. sakei</i>	89.81 ± 1.00d	69.61 ± 0.58abc	69.57 ± 0.00ab	67.58 ± 0.89a
<i>Leuconostoc</i> spp.	89.67 ± 0.29d	66.56 ± 1.00bc	68.45 ± 0.58ab	61.89 ± 0.58a

Notes: Values are the average of three repetition ± SD. The experiment was repeated twice. Values in a row followed by different lowercase letters are significantly different at  $p \leq 0.05$ .

*Enterococcus faecalis*, instead, showed the highest bacterial growth reduction (GR) ( $90.12 \pm 1.53\%$ ) through macrodilution method at 10 µg of NCE in growth broth (Table 2). *E. coli* was confirmed as the less susceptible bacterium to NCE treatment also using macrodilution method.

### 3. Conclusion

Neem cake is also potentially useful as preservative of retail fresh meat. It is needed to develop a methodology to test its antimicrobial activity directly on meat. Then, its use for antimicrobial packaging should be investigated.

### Supplementary material

Experimental details relating to this article are available online.

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