

Study of the stimulant activity of SantEnergy™ Nu in a *C. elegans* model



S-930

Test institute: Biopolis S.L., 46980 Paterna, Spain

Sponsor: Mibelle AG Biochemistry, 5033 Buchs, Switzerland

INTRODUCTION

The aim of this study was to evaluate the stimulating / energizing effects of SantEnergy™ Nu in the model organism *Caenorhabditis elegans* (*C. elegans*), a nematode known to be stimulated by caffeine treatment. Once treated with caffeine or other stimulants, *C. elegans* typically performs more movement bends per time in comparison to untreated nematodes ("mobility rate"). Furthermore, stimulated nematodes show a longer "endurance" ("dispersion rate").

STUDY DESIGN

Test samples

Besides SantEnergy™ Nu, pure caffeine was used as positive control. A stock solution was prepared for each test sample with 50% of DMSO solvent. This stock was dissolved in an ultrasonic bath at 40°C for 10 minutes. Each compound was added to the surface of standard Nematode Growth Medium (NGM) plates at a final DMSO concentration of 0.05% (DMSO was also added on control plates at the same concentration).

The information contained in this publication is provided in good faith and is based on our current knowledge. No legally binding promise or warranty regarding the suitability of our products for any specific use is made. These statements have not been evaluated by the Food and Drug Administration. This product is not intended to diagnose, treat, cure or prevent any disease. Mibelle AG Biochemistry will not assume any expressed or implied liability in connection with any use of this information. No part of this publication may be reproduced in any matter without the prior written permission of Mibelle AG Biochemistry.

Locomotion stimulation test

The experiments were performed using the *C. elegans* wild type strain N2. Synchronized nematodes were cultured in NGM plates or NGM supplemented with SantEnergy™ Nu or caffeine as positive control. When worms reached young adult stage, mobility and dispersion rate were measured for each culture condition. For mobility rate, the number of body bends (in form of sinusoidal waves, Figure 1) produced by individual worms for 40 seconds was counted using a stereomicroscope. A total of 25 nematodes per condition have been scored.

For dispersion rate, worms were placed at the center of the plate and, after 2 minutes, the number of worms in each area / zone (1, 2 or 3) was scored (Figure 1). Typically, most untreated worms stay in zone 1. Three independent assays were performed.

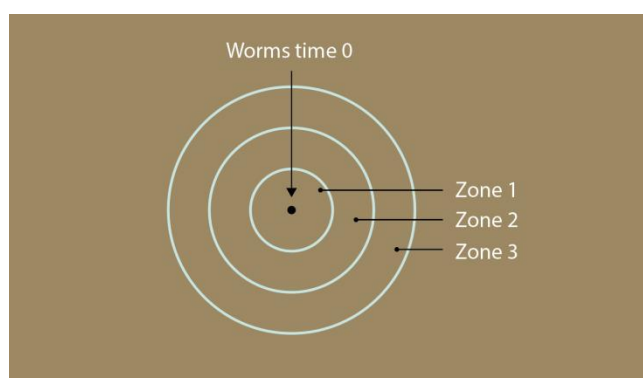


Figure 1: Locomotion activity in stimulated *C. elegans* nematodes: mobility (left) and dispersion rate (right)

RESULTS

Mobility rate

Nematodes treated with 1000 µg/ml SantEnergy™ Nu perform significantly more body bends (+ 6.9 %) than untreated nematodes ($p < 0.001$), clearly demonstrating a stimulation effect. The activity level mediated by SantEnergy™ Nu is comparable to the activity of 100 µg/ml pure caffeine (Figure 2).

The information contained in this publication is provided in good faith and is based on our current knowledge. No legally binding promise or warranty regarding the suitability of our products for any specific use is made. These statements have not been evaluated by the Food and Drug Administration. This product is not intended to diagnose, treat, cure or prevent any disease. Mibelle AG Biochemistry will not assume any expressed or implied liability in connection with any use of this information. No part of this publication may be reproduced in any matter without the prior written permission of Mibelle AG Biochemistry.

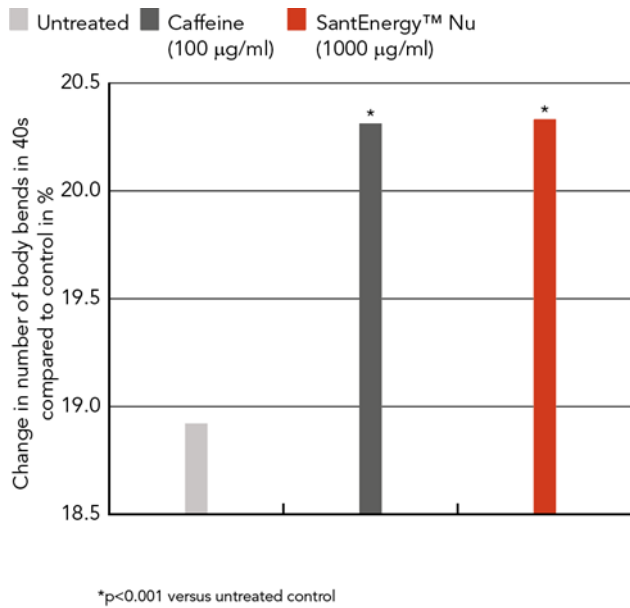


Figure 2. Improved mobility rate mediated by 1000 µg/ml SantEnergy™ Nu

Dispersion rate

In comparison to untreated nematodes, nematodes treated with 200 µg/ml SantEnergy™ Nu showed an increased presence in zone 2 and 3 (Figure 3), indicating next to the stimulation effect also an endurance effect.

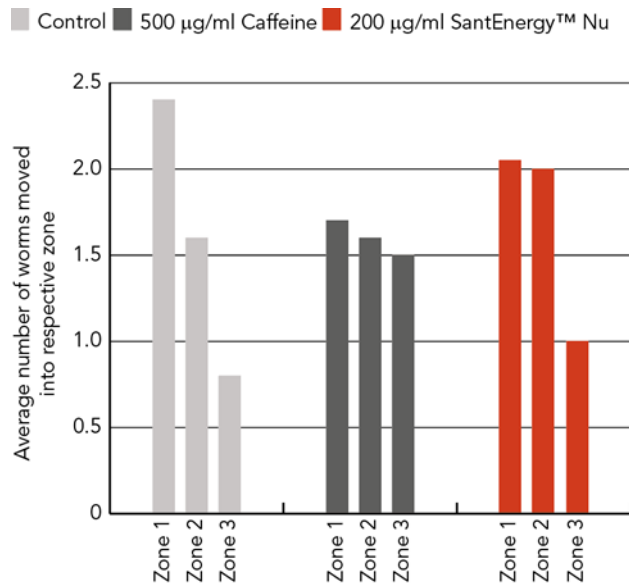


Figure 3. Improved dispersion rate mediated by 200 µg/ml SantEnergy™ Nu

The information contained in this publication is provided in good faith and is based on our current knowledge. No legally binding promise or warranty regarding the suitability of our products for any specific use is made. These statements have not been evaluated by the Food and Drug Administration. This product is not intended to diagnose, treat, cure or prevent any disease. Mibelle AG Biochemistry will not assume any expressed or implied liability in connection with any use of this information. No part of this publication may be reproduced in any matter without the prior written permission of Mibelle AG Biochemistry.