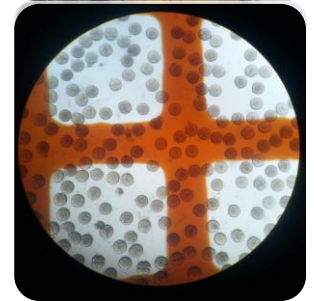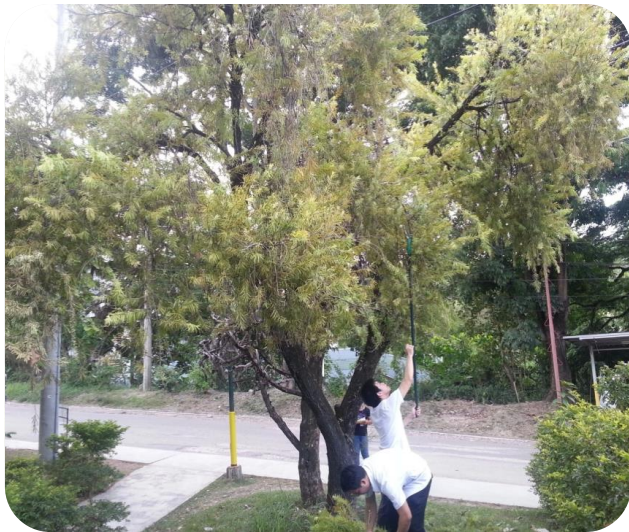# Antidevelopmental effects of *Callistemon viminalis* (Weeping bottlebrush) leaf extract on the early development of *Tripneustes gratilla* L. (Sea urchin) embryos

Cercado, G., Arnaiz, K., Dy, A., Joshi, G., Filipinas, N., Lao, M., Moscoso, R., Pasco, D., Quirante, F., Rodriguez, R., Sy, R.

# PROJECT DESCRIPTION

➢ **Cancer is a leading cause of mortality and morbidity in the world**

➢ **Chemotherapy – expensive and hard to avail**

➢ **Studies have been conducted on *Callistemon* species which have been proven to possess cytotoxic effects**

➢ **No documented antidevelopmental activity of *Callistemon viminalis* on sea urchin embryos**

# GENERAL OBJECTIVE

➢ **To determine if *Callistemon viminalis* (weeping bottlebrush) leaf extract exhibit anti-developmental effects on the early development of *Tripneustes gratilla* L. (sea urchin) embryos using seawater as control**

# SPECIFIC OBJECTIVES

➢ **To determine and compare the proportion of embryos present in each of the developmental stage and the proportion of embryos with abnormalities in seawater and in the different leaf extract concentrations**

➢ **To determine the proportion of embryos that has reached blastula stage 24 hours post-fertilization exposed to the different leaf extract concentrations and seawater**

# METHODOLOGY



SPAWNING INDUCTION
(injection of 0.55M KCl)

COLLECTION OF
GAMETES

DILUTION
with 250mL seawater

EMBRYO SUSPENSION:
140mL male gametes +
350mL female gametes

Female Gametes

Male Gametes

# METHODOLOGY

## IV. Monitoring and Evaluation

### B. Counting Developmental Activity
### A. Time Intervals

Evaluation of embryos undergoing the following stages:
- every 30 minutes for 4 hours post-fertilization and
- 1-cell stage
- 2-cell stage
- every 2 hours for the next 4 hours post-fertilization (24 hours monitoring)
- 4-cell stage
- 8-cell stage
- 32-cell stage
- morula stage
- blastula stage
- number of embryos with morphological abnormalities

$$Percent\ Composition\ (\%) = \frac{number\ of\ embryo\ in\ a\ cell\ stage}{total\ number\ of\ embryos} \times 100$$

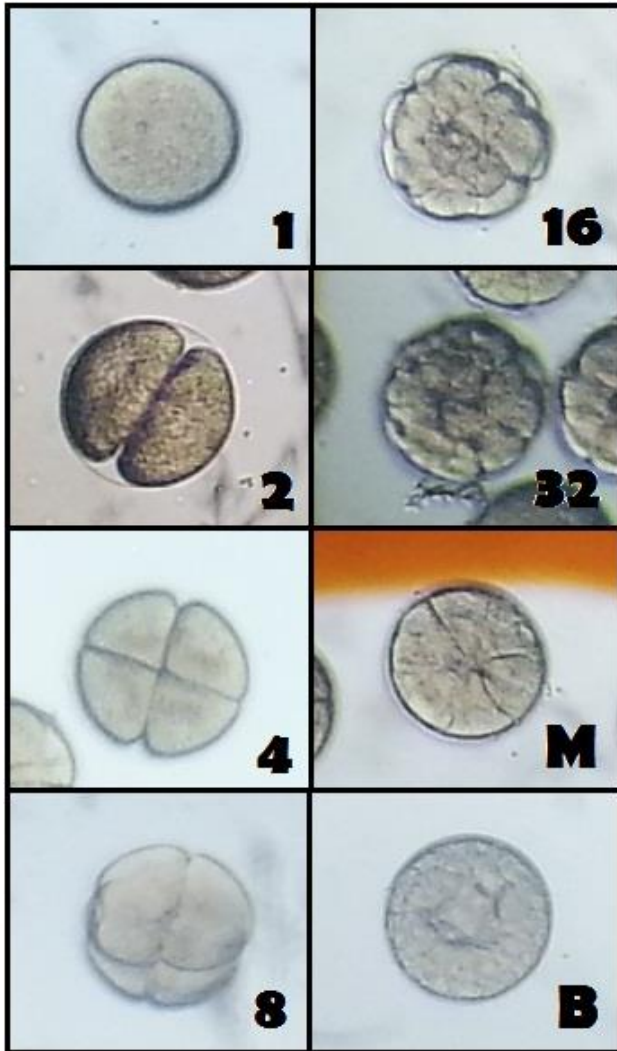# EMBRYOLOGIC DEVELOPMENT OVER 24 HOURS -- SEAWATER
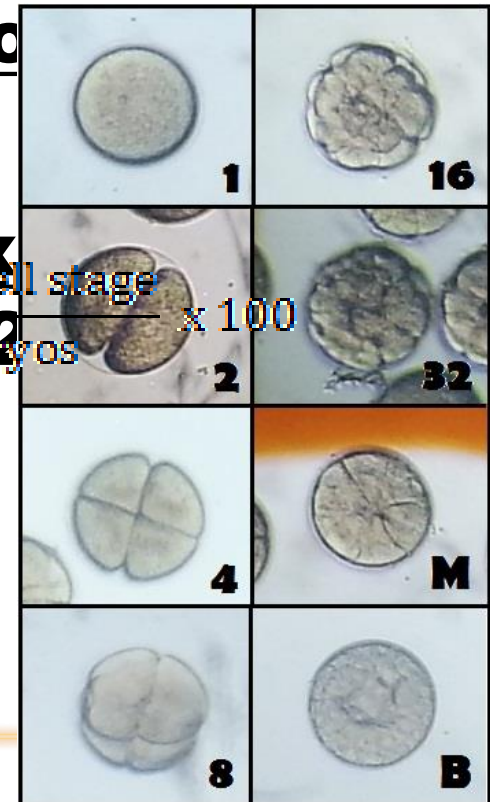
# EMBRYOLOGIC DEVELOPMENT OVER 24 HOURS -- 5% C. *viminalis* LEAF EXTRACT CONCENTRATION
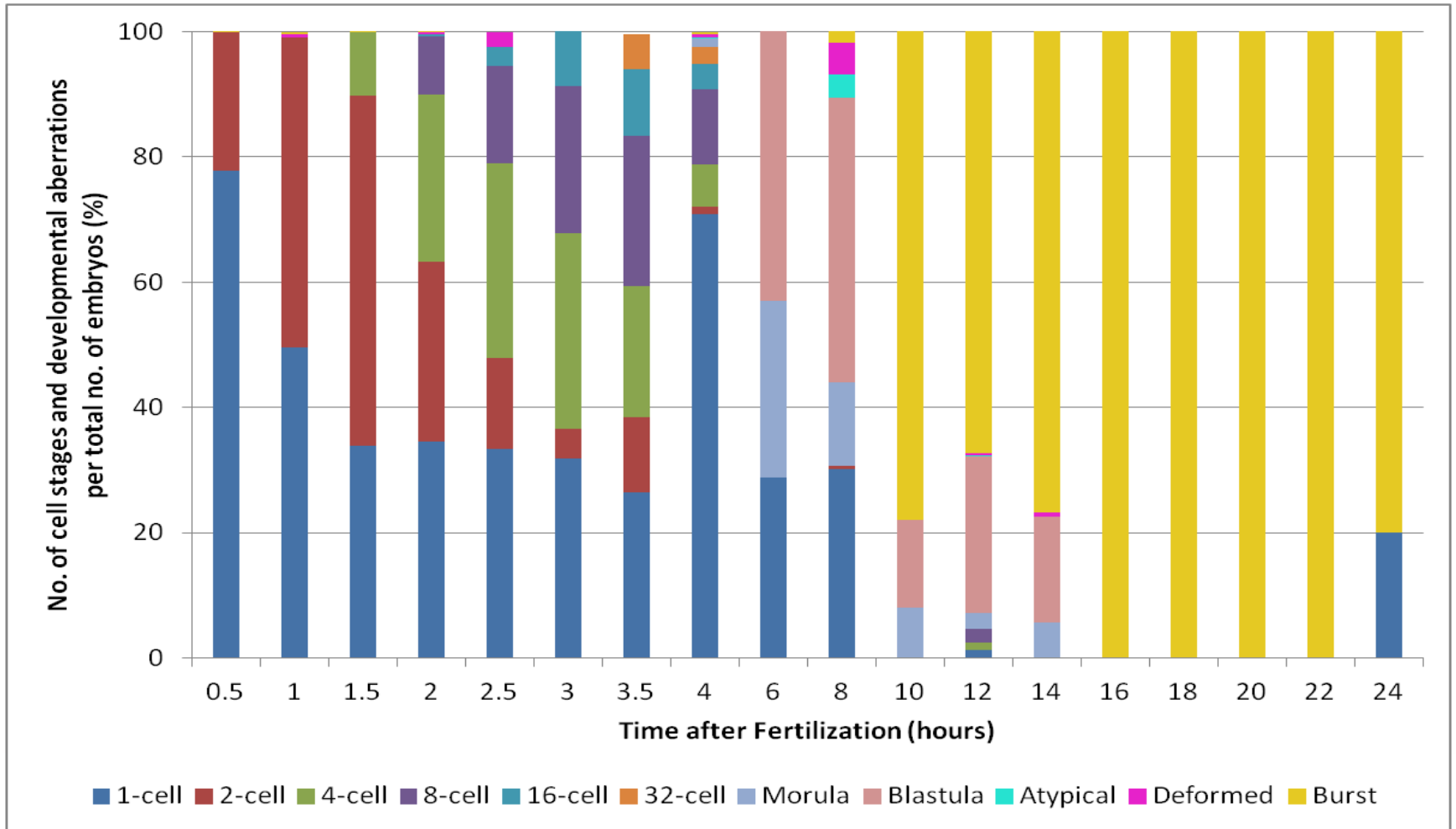
# EMBRYOLOGIC DEVELOPMENT OVER 24 HOURS -- 10% C. *VIMINALIS* LEAF EXTRACT CONCENTRATION
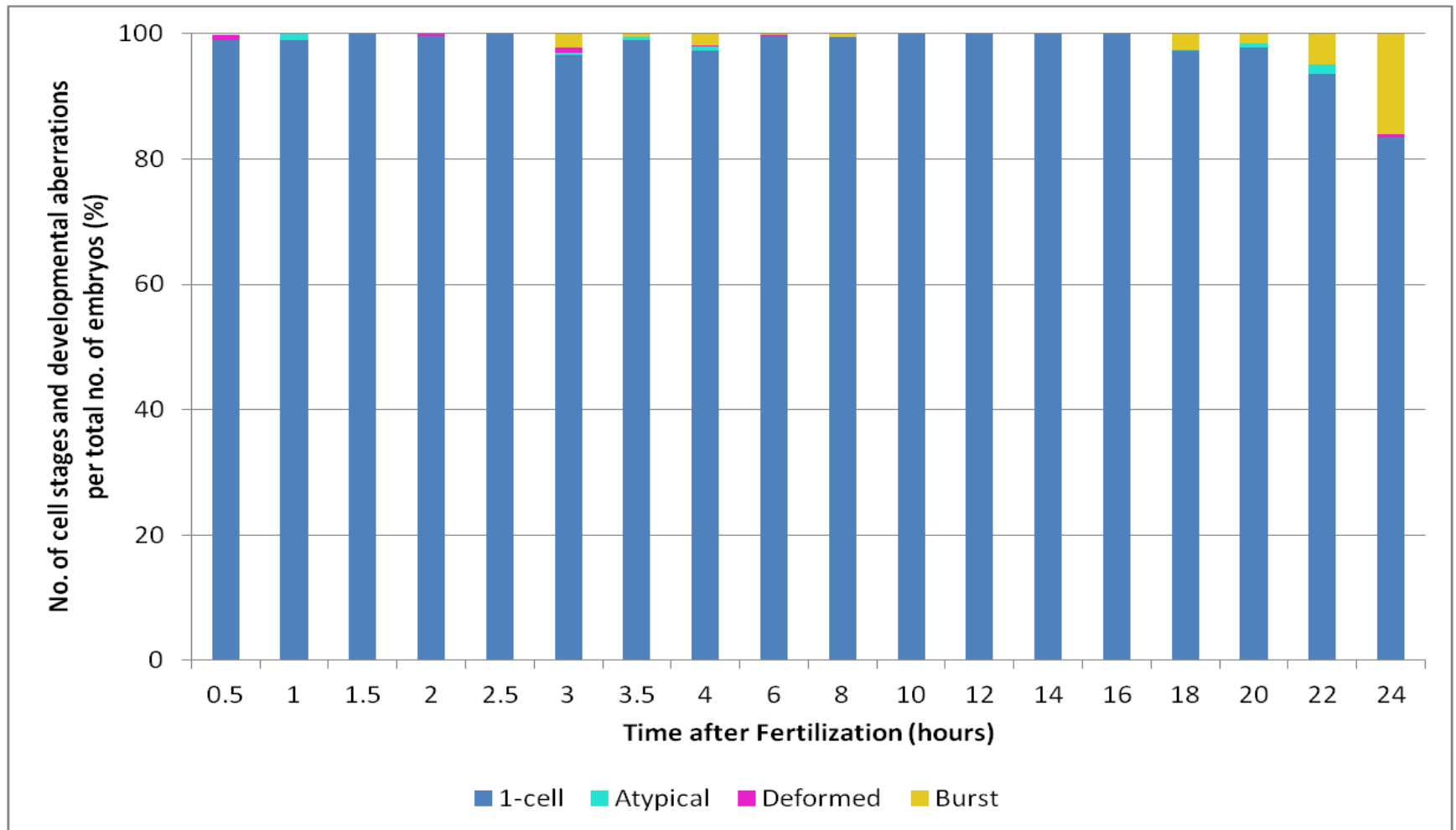
# EMBRYOLOGIC DEVELOPMENT: SEAWATER VS. 5% CONCENTRATION



| Cell Stage | Time of Development (hr) | |
| --- | --- | --- |
| | **SEAWATER** | **5% conc.** |
| 2-cell | 0.5 - 1 | 1 - 1.5 |
| 4-cell | 1.5 - 2 | 2 - 3 |
| 8-cell | 2 - 2.5 | 2.5 - 3.5 |
| 16-cell | 2.5 - 3 | 3 - 3.5 |
| 32-cell | 3 - 3.5 | 3.5 - 4 |
| Morula | 3 - 6 | 6 - 10 |
| Blastula | 4 -8 | 6 -14 |

✓ **5% conc. – delayed development**

# PERCENTAGE OF EMBRYOS REACHING BLASTULA STAGE 24 HOURS POST-FERTILIZATION



Treatments (Seawater and concentrations of *C.viminalis* leaf exrtract)
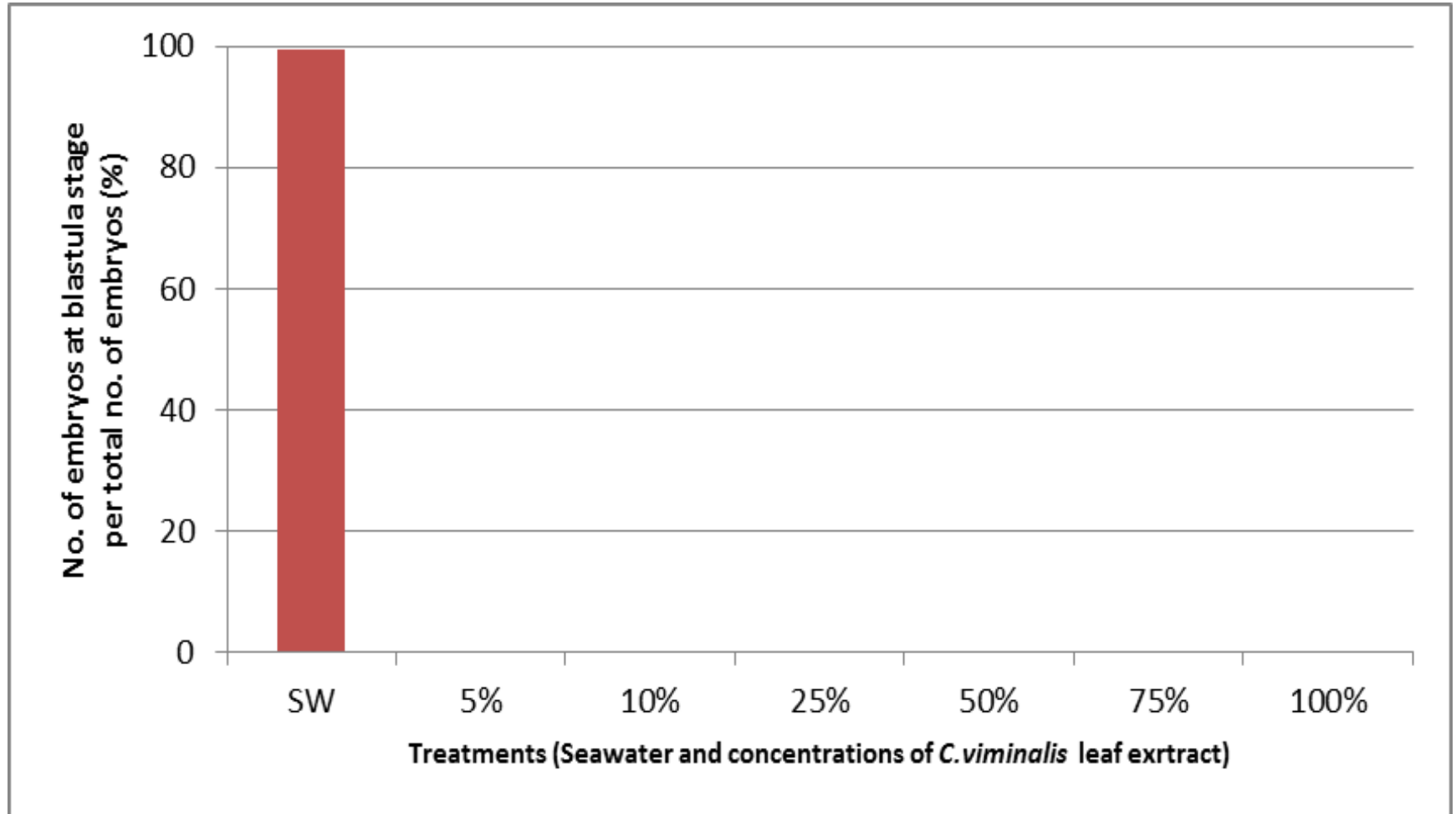
# MORPHOLOGICAL ABNORMALITIES
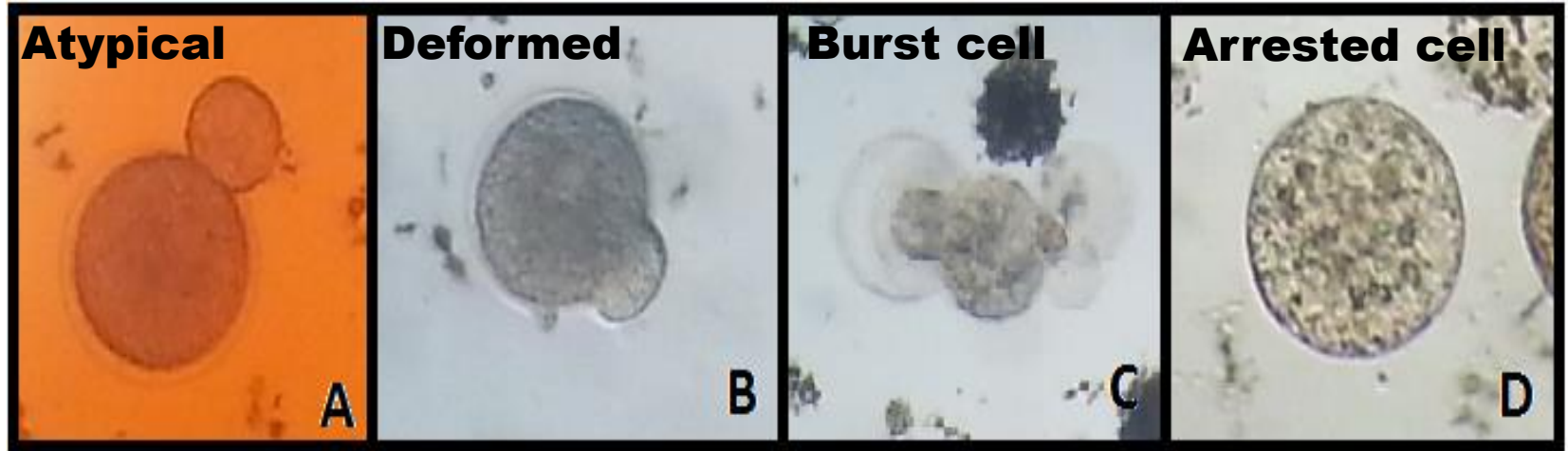
# CONCLUSION

➤ **The leaf extract of *Callistemon viminalis* exhibit antidevelopmental effects on the sea urchin embryos: developmental delay; cell arrest; presence of deformities, burst, and atypical cells**

➤ **99.6% of embryos in seawater while none of the embryos in the different extract concentrations reached blastula stage 24-hours post-fertilization**

# RECOMMENDATION

➢ **Isolation and characterization of the specific bioactive compounds responsible for the antidevelopmental effects of the leaf of *Callistemon viminalis.***

➢ **Testing of other parts of C. *viminalis* for similar antidevelopmental properties.**

➢ **Testing on various cancer cell lines.**

# REFERENCES

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➢ Abdelhady MIS, Motaal AA, Beerhues L. Total phenolic content and antioxidant activity of standardized extracts from leaves and cell cultures of three *callistemon* species. Am J Plant Res. 2011: 2, 847-850.

➢ Kumar PP, Kumaravel S, Lalitha C. Screening of antioxidant activity, total phenolics and GC-MS study of vitex negundo. African Journal of Biochemistry Research. 2010 July: 4(7): 191-195. Available from: http://academicjournals.org/journal/AJBR/article-full-text-pdf/743A94611519 (accessed 15 February 2014)

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