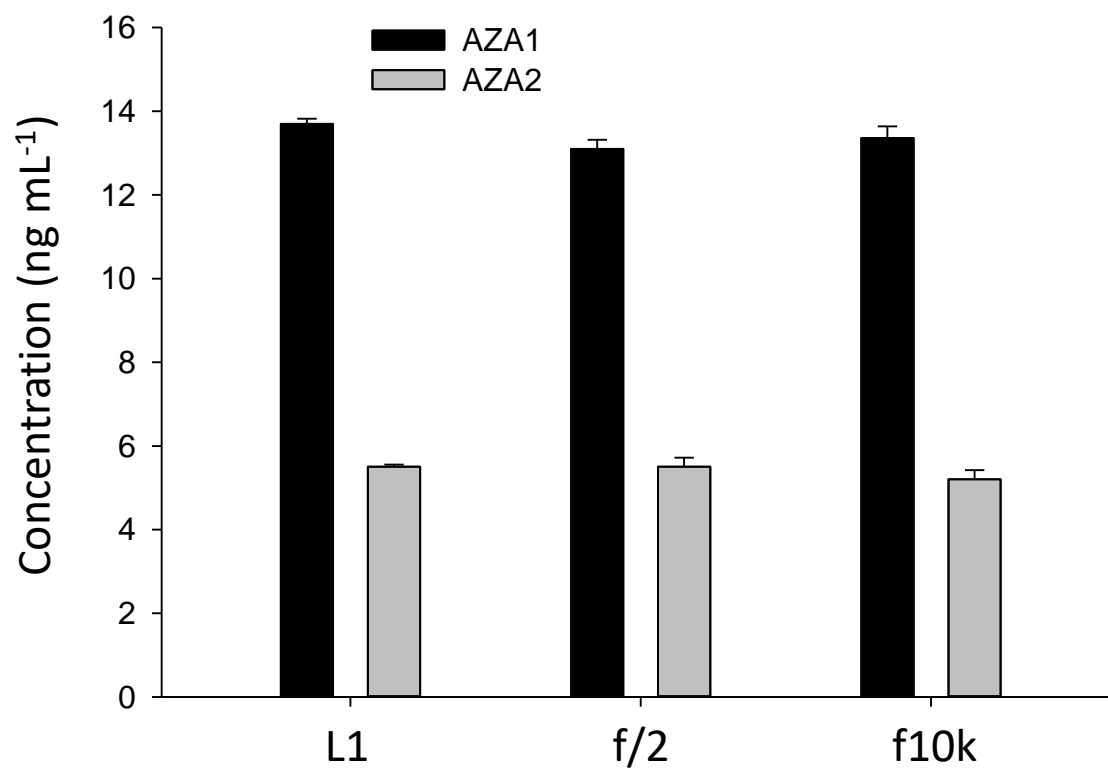


# Effects of temperature, growth media, and photoperiod on growth and toxin production of *Azadinium spinosum*

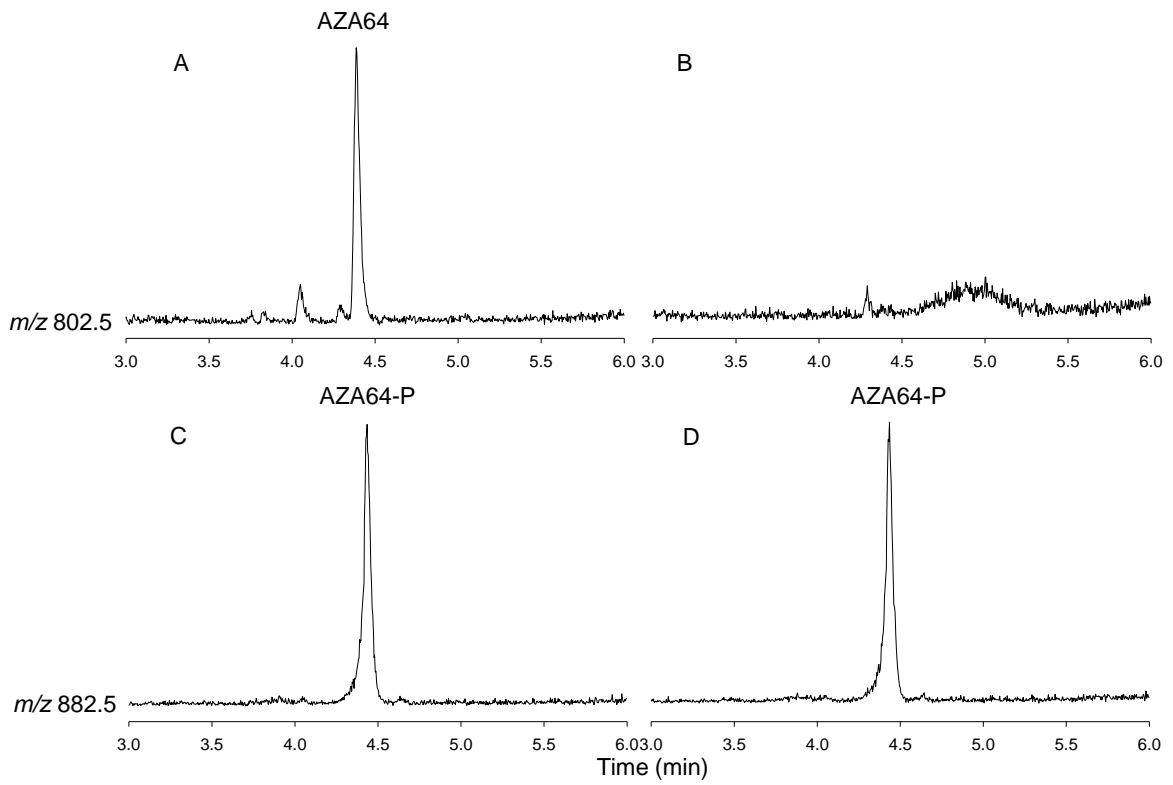
Jane Kilcoyne, Amy McCoy, Stephen Burrell, Bernd Krock and Urban Tillmann

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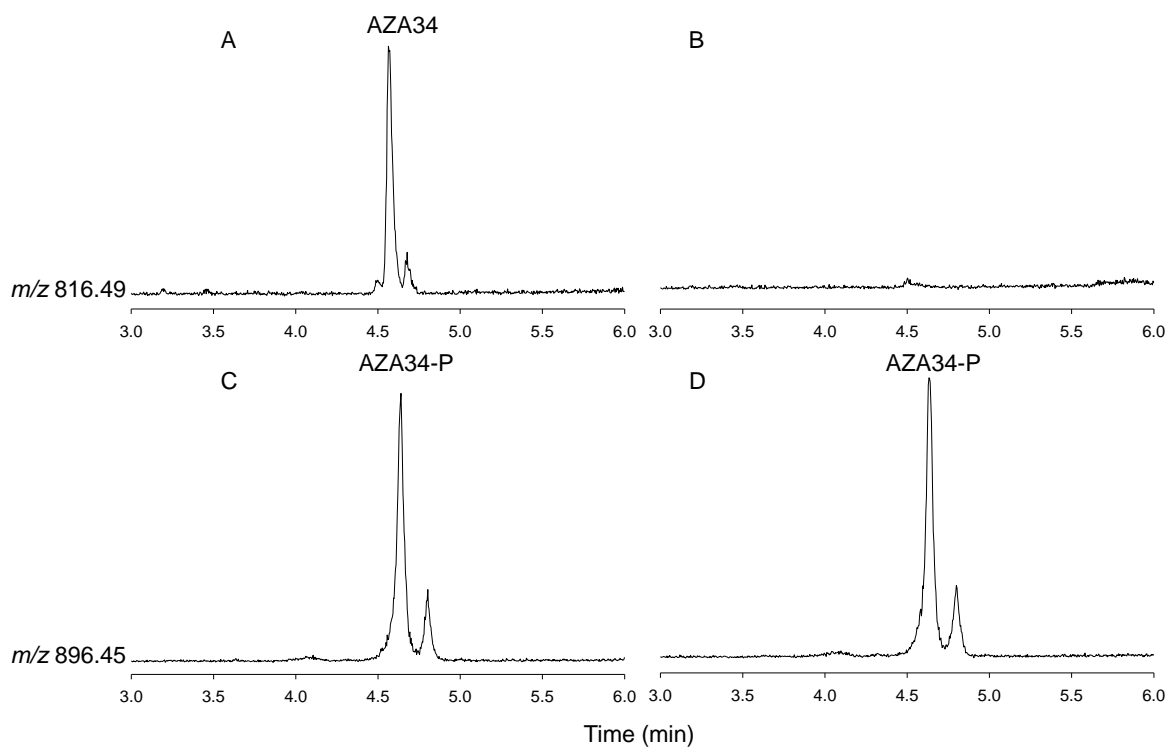
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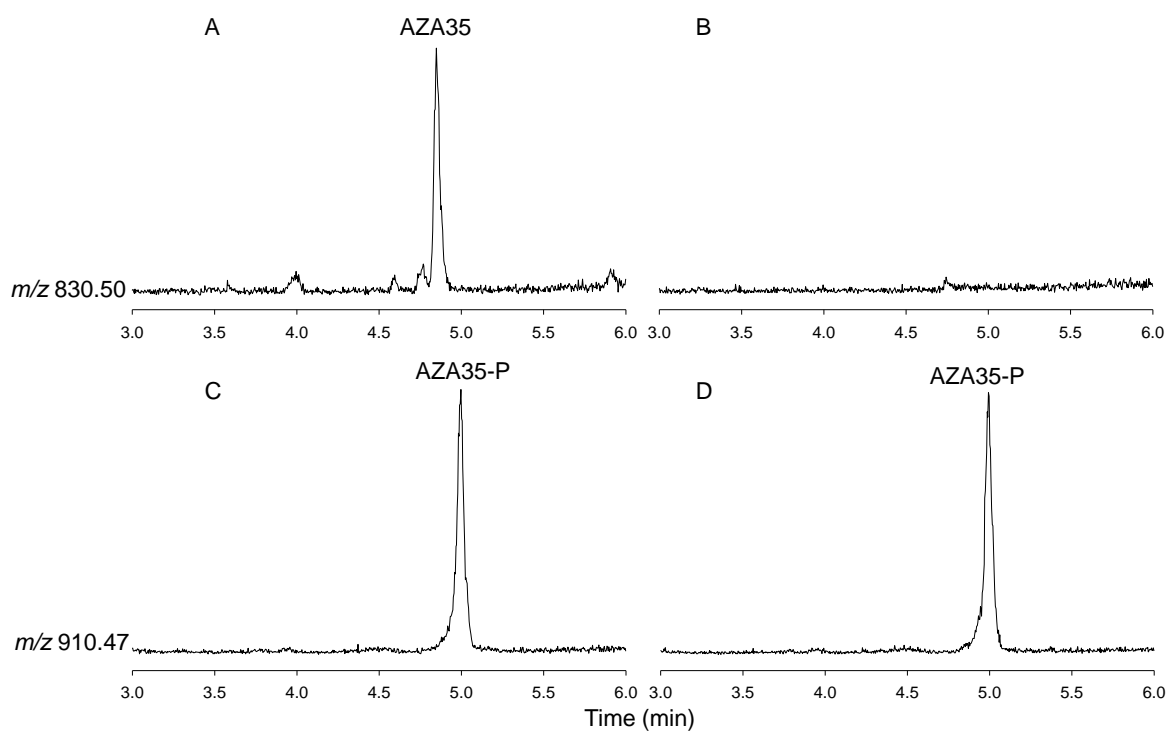
**Figure S1.** LC-MS analysis of L1, f/2 and f10k media spiked with a CRM containing AZA1 and -2.



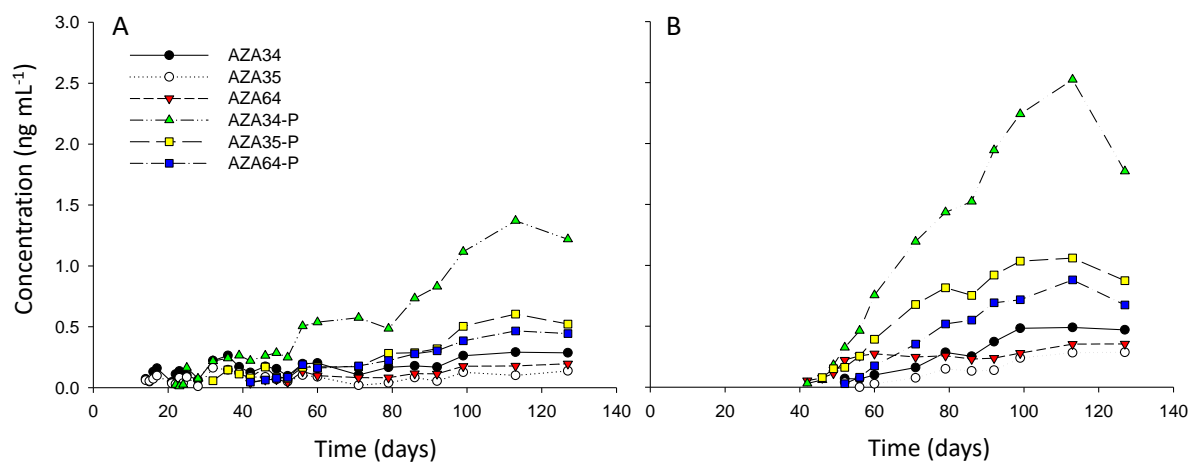
**Figure S2.** LC-MS analysis of *A. spinosum* HP20 resin extract showing chromatogram of A) AZA64, B) AZA64 after treatment with periodate, C) AZA64-P and D) AZA64-P after treatment with periodate.



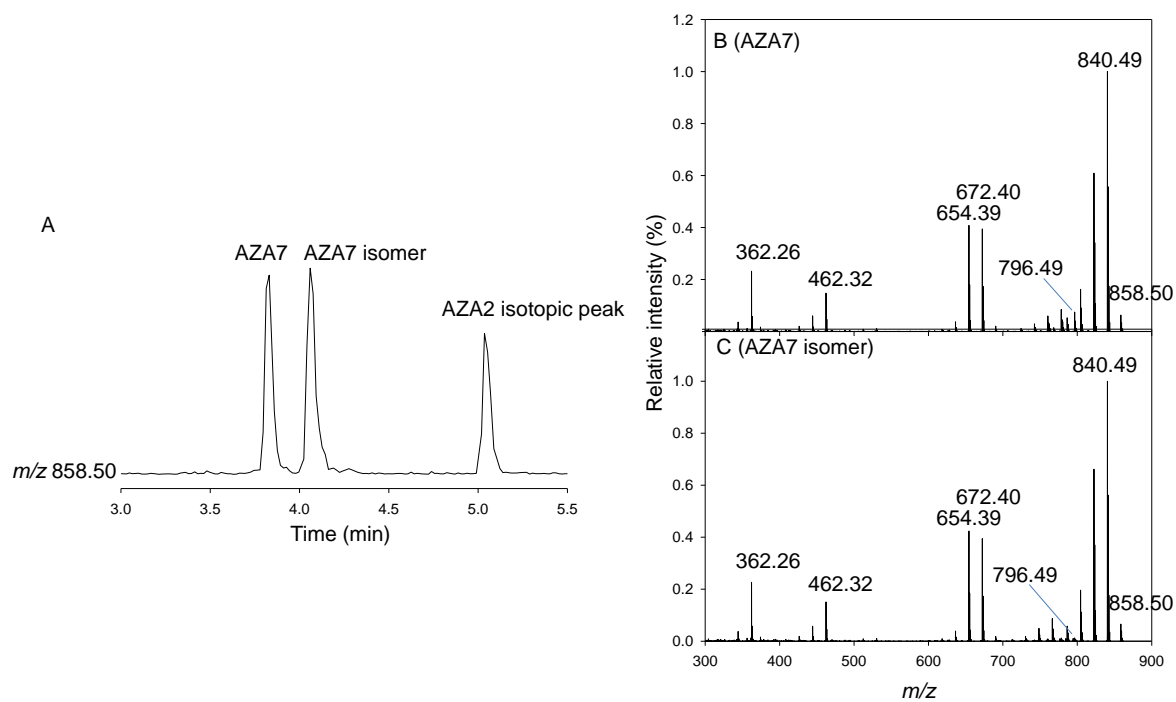
**Figure S3.** LC-MS analysis of *A. spinosum* HP20 resin extract showing chromatogram of A) AZA34, B) AZA34 after treatment with periodate, C) AZA34-P and D) AZA34-P after treatment with periodate.



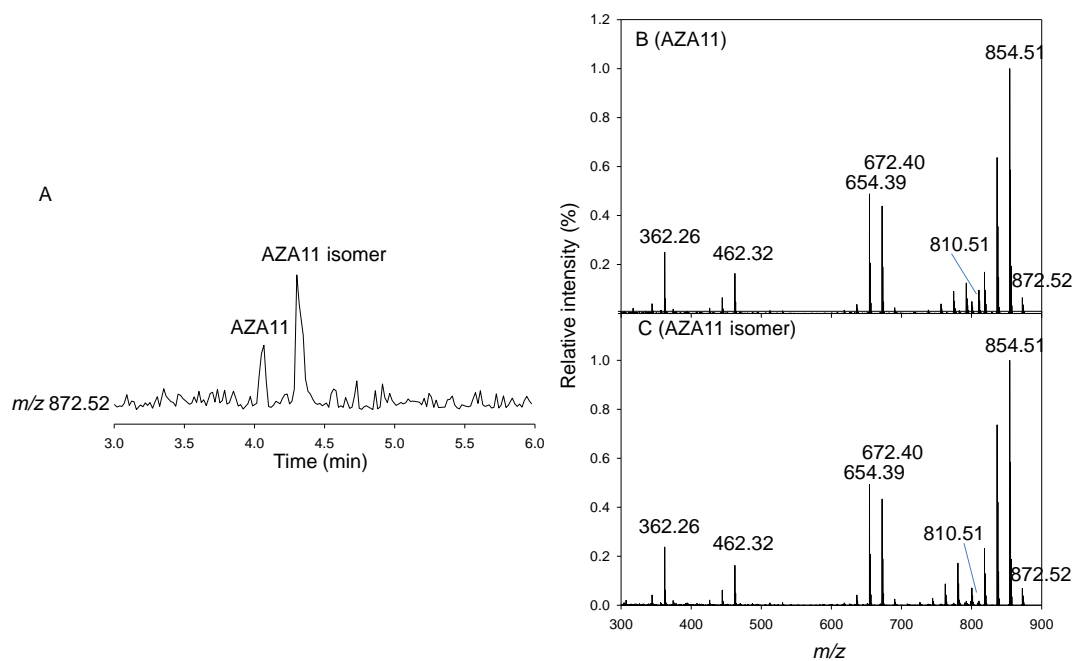
**Figure S4.** LC-MS analysis of *A. spinosum* HP20 resin extract showing chromatogram of A) AZA35, B) AZA35 after treatment with periodate, C) AZA35-P and D) AZA35-P after treatment with periodate.



**Figure S5.** Figure 2 zoomed in - *A. spinosum* growth curves at A) 18 °C and B) 10 °C in the 5 L culture flasks showing concentration changes for AZA34, -35, -64 and their phosphorylated conjugates.

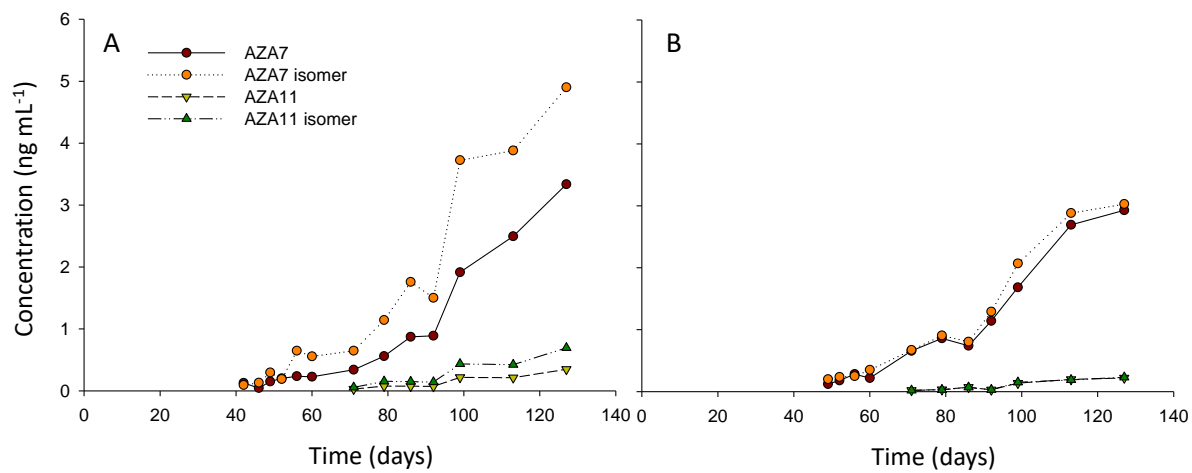


**Figure S6.** LC-MS analysis of *A. spinosum* HP20 extract showing A) chromatogram of AZA7 and isomer, B) AZA7 mass spectrum and C) AZA7 isomer mass spectrum (CE=50 V).

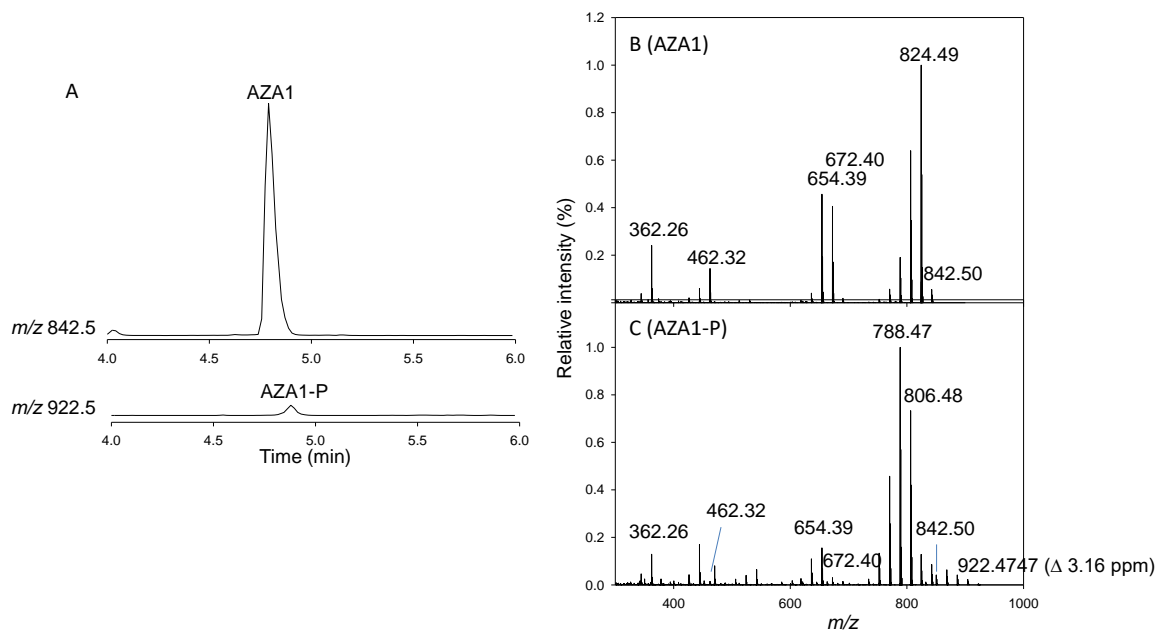


**Figure S7.** LC-MS analysis of *A. spinosum* HP20 extract showing A) chromatogram of AZA11 and isomer, B) AZA11 mass spectrum and C) AZA11 isomer mass spectrum (CE=50 V).

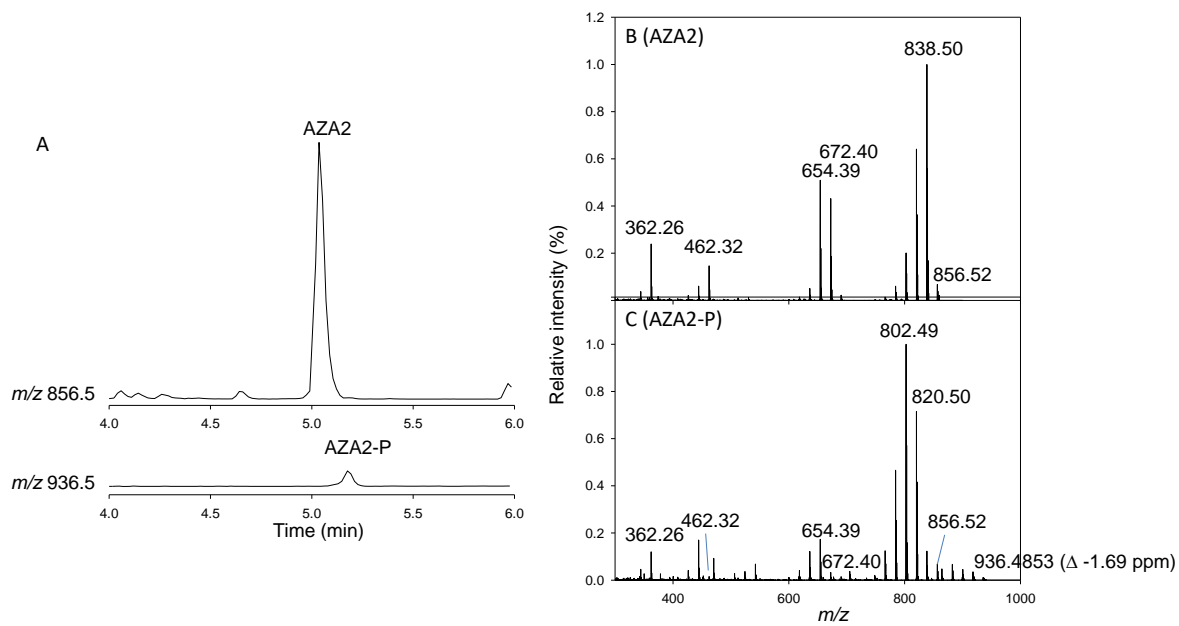




**Figure S8.** Figure 2 zoomed in - *A. spinosum* growth curves at A) 18 °C and B) 10 °C in the 5 L culture flasks showing concentration changes for AZA7, -11 and their isomers.



**Figure S9.** LC-MS analysis of a SPATT extract showing A) chromatogram of AZA1 and AZA1-P, B) AZA1 mass spectrum and C) AZA1-P mass spectrum (CE=50 V).



**Figure S10.** LC-MS analysis of a SPATT extract showing A) chromatogram of AZA2 and AZA2-P, B) AZA2 mass spectrum and C) AZA2-P mass spectrum (CE=50 V).

**Table S1.** Cell counts and quotas in the 18 °C culture flask

| Time                       | Growth Phase | Cell counts<br>(cells mL <sup>-1</sup> ) | AZA1<br>(fg cell <sup>-1</sup> ) | AZA2<br>(fg cell <sup>-1</sup> ) | AZA33<br>(fg cell <sup>-1</sup> ) |
|----------------------------|--------------|--|----------------------------------|----------------------------------|-----------------------------------|
| 0                          | E            | 14,464                                   |                                  |                                  |                                   |
| 1                          | E            | 15,692                                   |                                  |                                  |                                   |
| 2                          | E            | 24,412                                   |                                  |                                  |                                   |
| 3                          | E            | 37,000                                   |                                  |                                  |                                   |
| 4                          | E            | 53,250                                   | 8.0                              | 7.2                              | 4.5                               |
|                            |              |  | 8.0                              | 7.2                              | 4.5                               |
| 9                          | S            | 163,667                                  | 15.1                             | 11.0                             | 8.5                               |
| 10                         | S            | 165,667                                  | 14.8                             | 10.7                             | 8.2                               |
| 11                         | S            | 170,000                                  | 16.6                             | 12.3                             | 11.0                              |
| Average                    |              |  | 15.5 ± 0.9                       | 11.3 ± 0.9                       | 9.2 ± 1.5                         |
| Fold increase from E phase |              |  | 1.9                              | 1.6                              | 2.0                               |
| 14                         | LS           | 173,333                                  | 50.0                             | 27.9                             | 11.4                              |
| 15                         | LS           | 177,667                                  | 47.2                             | 31.7                             | 20.3                              |
| 16                         | LS           | 170,668                                  | 41.8                             | 26.2                             | 19.3                              |
| 17                         | LS           | 180,666                                  | 42.4                             | 24.3                             | 17.6                              |
| Average                    |              |  | 43.8 ± 3.9                       | 27.4 ± 3.1                       | 19.1 ± 4.0                        |
| Fold increase from S phase |              |  | 2.8                              | 2.4                              | 2.1                               |

E = exponential, S = stationary, LS = late stationary

**Table S2.** Cell counts and quotas in the 10 °C culture flask

| Time                       | Growth Phase | Cell counts<br>(cells mL <sup>-1</sup> ) | AZA1<br>(fg cell <sup>-1</sup> ) | AZA2<br>(fg cell <sup>-1</sup> ) | AZA33<br>(fg cell <sup>-1</sup> ) |
|----------------------------|--------------|--|----------------------------------|----------------------------------|-----------------------------------|
| 0                          | E            | 14,214                                   |                                  |                                  |                                   |
| 1                          | E            | 14,138                                   |                                  |                                  |                                   |
| 2                          | E            | 13,500                                   |                                  |                                  |                                   |
| 3                          | E            | 12,719                                   |                                  |                                  |                                   |
| 4                          | E            | 12,029                                   |                                  |                                  |                                   |
| 9                          | E            | 19,095                                   | 54.4                             | 40.6                             | 19.4                              |
| 10                         | E            | 16,520                                   | 41.6                             | 21.4                             | 14.6                              |
| 11                         | E            | 19,091                                   | 30.6                             | 21.8                             | 17.2                              |
| 14                         | E            | 26,438                                   | 68.9                             | 49.3                             | 19.7                              |
| 15                         | E            | 26,733                                   | 47.5                             | 34.8                             | 23.2                              |
| 16                         | E            | 29,857                                   | 39.7                             | 37.6                             | 24.0                              |
| 17                         | E            | 32,769                                   | 60.0                             | 43.0                             | 30.0                              |
| 21                         | E            | 47,222                                   | 55.8                             | 47.5                             | 20.2                              |
| 22                         | E            | 50,250                                   | 54.9                             | 49.7                             | 22.3                              |
| 23                         | E            | 62,857                                   | 77.6                             | 61.5                             | 26.3                              |
| 24                         | E            | 73,167                                   | 82.0                             | 58.5                             | 25.8                              |
| 25                         | E            | 76,000                                   | 74.9                             | 59.1                             | 29.1                              |
| 28                         | E            | 102,000                                  | 91.9                             | 76.5                             | 37.4                              |
|                            |              |  | 60.0 ± 18.2                      | 46.3 ± 15.7                      | 23.8 ± 6.1                        |
| 32                         | S            | 174000                                   | 71.4                             | 70.7                             | 32.3                              |
| 36                         | S            | 175000                                   | 99.1                             | 102.5                            | 51.0                              |
| Average                    |              |  | 85.3                             | 86.6                             | 41.6                              |
| Fold increase from E phase |              |  | 1.4                              | 1.9                              | 1.8                               |
| 39                         | LS           | 166000                                   | 135.5                            | 127.0                            | 50.3                              |
| 42                         | LS           | 175000                                   | 130.7                            | 127.9                            | 43.3                              |
| 46                         | LS           | 167000                                   | 131.5                            | 118.5                            | 41.6                              |
| 49                         | LS           | 170000                                   | 115.9                            | 108.8                            | 33.4                              |
| 52                         | LS           | 164000                                   | 158.9                            | 162.6                            | 31.3                              |
| Average                    |              |  | 134.5 ± 15.5                     | 129.0 ± 20.3                     | 40.0 ± 7.7                        |
| Fold increase from S phase |              |  | 1.6                              | 1.5                              | 1.0                               |

E = exponential, S = stationary, LS = late stationary

**Table S3.** Differences between L1, f/2 and f10k media.

|   | fold difference |             |
|---|-----------------|-------------|
|   | L1 vs f10k      | f/2 vs f10k |
| Nitrates  | 4.5             | 4.5         |
| Phosphates  | 4.5             | 4.5         |
|   |                 |             |
| FeCl <sub>3</sub> .6H <sub>2</sub> O                | 4.5             | 4.5         |
| CuSO <sub>4</sub> .5H <sub>2</sub> O                | 1.2             | 4.5         |
| ZnSO <sub>4</sub> .7H <sub>2</sub> O                | 4.7             | 4.5         |
| CoCl <sub>2</sub> .6H <sub>2</sub> O                | 5.3             | 4.5         |
| MnCl <sub>2</sub> .2H <sub>2</sub> O                | 4.5             | 4.5         |
| Na <sub>2</sub> MoO <sub>4</sub> .2H <sub>2</sub> O | 14.3            | 4.5         |
| H <sub>2</sub> SeO <sub>3</sub>                     | 1.1             | none in f/2 |
| Na <sub>2</sub> EDTA.2H <sub>2</sub> O              | 1.6             | 1.6         |
| NiSO <sub>4</sub> .6H <sub>2</sub> O                | none in f10k    | none in f/2 |
| Na <sub>3</sub> VO <sub>4</sub>                     | none in f10k    | none in f/2 |
| K <sub>2</sub> CrO <sub>4</sub>                     | none in f10k    | none in f/2 |
|   |                 |             |
| Thiamine  | same            | same        |
| Biotin  | same            | same        |
| B12   | same            | same        |

Red = lower amounts in f10k, Green = higher amounts in f10k

**Table S4.** Relative (%) concentrations of AZAs to AZA1 in the harvested culture (5 L flasks) and SPATT extracts.

|              | Culture 10 °C | Culture 18 °C | SPATT (0 m) | SPATT (5 m) |
|--------------|---------------|---------------|-------------|-------------|
| AZA2         | 110.3         | 112.1         | 41.1        | 38.4        |
| AZA33        | 10.3          | 21.2          | 4.6         | 4.7         |
| AZA34        | 24.1          | 12.1          | 1.0         | 0.7         |
| AZA35        | 10.3          | 9.1           | 0.1         | 0.0         |
| AZA11        | 3.4           | 6.1           | 0.4         | 0.3         |
| AZA11 isomer | 5.2           | 12.1          | 0.1         | 0.1         |
| AZA7         | 82.8          | 124.2         | 4.7         | 0.1         |
| AZA7 isomer  | 84.5          | 163.6         | 1.1         | 1.0         |
| AZA34-P      | 62.1          | 63.6          | 0.6         | 0.3         |
| AZA35-P      | 20.7          | 21.2          | 0.1         | 0.1         |
| AZA64        | 10.3          | 9.1           | 12.8        | 8.7         |
| AZA64-P      | 17.2          | 12.1          | 3.1         | 3.5         |
| AZA1-P       | n.d.          | n.d.          | 6.5         | 5.9         |
| AZA2-P       | n.d.          | n.d.          | 2.4         | 3.9         |

n.d. = not detected