SUSTAINABLE DEVELOPMENT OF AMOMUM VILLOSUM: A SYSTEMATIC INVESTIGATION ON THREE DIFFERENT PRODUCTION MODES

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Abstract

Background: Amomum Villosum (A. Villosum), called Chunsharen in Chinese, is widely used in treating gastrointestinal disease. Its clinical benefits have been confirmed by both in vitro and in vivo studies. Facing the shortage of wild A. Villosum, artificial cultivating and natural fostering have been practiced in recent years. Therefore, it would be wondered whether the three different types of A. Villosum are comparable or not, particularly the herbal qualities, technological challenges, ecological impacts and economic benefits.

Material and methods: In this study, we combined quality research by using GC-MS, and field investigation to provide a systematic assessment about the three types of A. Villosum from these four aspects.

Results: It found that the wild type had low output and was in an endangered situation. The artificial cultivation had larger agricultural area with higher productivity, but faced the ecological challenges. Lastly, the natural fostering type generated the highest economic benefit and relatively low ecological impact. In addition, the natural fostering type had relatively better quality than the other types.

Conclusion: Therefore, it suggests that natural fostering can be applied for long-term sustainable development of A. Villosum.

Key words: production mode; Amomum Villosum; natural fostering; artificial cultivating; quality evaluation

Introduction

Amomum Villosum (A. Villosum), belonging to the genus Zingiberaceae, is native to Guangdong Province of China, with more than 1,300 years of medical application history (Duang et al., 2009). Called as Chunsharen in Chinese, it is a very important Chinese herb distributed in southeast and east Asia (Li et al., 2011; Lee et al., 2012; Zhao et al., 2013; Zhao et al., 2011). The main chemical components in A. Villosum are camphor, borneol, borneol acetate, copaene, etc, and these main ingredients are very high in content (Chen et al., 2014; Yin et al., 2008; Wang & Situ, 2010; Zhang et al., 2011). Research found that the pharmacological activities of A. Villosum include direct protective effect on gastric mucosal barrier (Jafri et al., 2001; Chinese Pharmacopoeia Commission, 2010; Zhao et al., 2011), treatment on growth retardation during adolescence (Lee et al., 2012), anti-inflammation, analgesic and anti-diarrhea (Zhao et al., 2009), anti-platelet aggregation, and prolonging coagulation time (Zhang & Shen, 2013). It is not only an important herb included in many prescriptions of traditional Chinese medicine (TCM), but also used for health food (Zhou, 1993). Therefore, A. Villosum is widely regarded as a kind of herbal resource that has high medical and economic values (Zhang et al., 2013a; Peng et al., 2012).

With the development of TCM industry, the demand on Chinese herb rapidly increased. Up to 80% of Chinese herbs come from continuous wild collection without scientific planning (Chen & Xiao, 2006). However, the natural resource is limited and hard to meet the speedily growing demand. As one of the most commonly used Chinese herbs, A. Villosum is facing huge market demand and cannot be supplied by wild collecting (Tang et al., 2012). In fact, the market demand of A. Villosum has grown to more than 2.2x10⁸ kg in 2008, but the total production in China was only about 1.6x10³ kg (Tang et al., 2012). Therefore, natural resource of A. Villosum has already been in the endangered state (Li & Wu, 2012), so artificial cultivating and natural fostering have been practiced in recent years for A. Villosum production to make up the shortage of A. Villosum.

Artificial cultivating uses artificial environment and techniques to produce herbs in a large scale (Li et al., 2015). On the contrary, natural fostering named as wild nursery or semi-imitational cultivation, emphasizes planting herbs in their natural habitats with limited human intervention (Li & Chen, 2007; Li et al., 2015; Li et al., 2012). As three different production modes are used for A. Villosum, comparative study on these three production modes began to attract the attention of researchers. For example, Zhou (1993) and Liu et al. (2006) introduced the ecological characteristics of A. Villosum in natural forest and tree plantation. Ducourtieux et al.
Preparation of sample extract

The dried sample powder (0.1 g, 40 mesh) and 3 mL ethyl acetate were transferred into 5 mL extraction vessels made of borosilicate glass. The microwave-assisted extraction was carried out in Multiwave 3000 (Anton Paar GmbH, Graz, Austria), which was performed at 100 W and 80 °C for 3 min. Then, the extract was compensated the loss of weight with extraction solvent and subsequently centrifuged at 5000 x g for 5 min. After centrifugation, the supernatant was filtered through a 0.45 μm filter for further analysis.

GC-MS analysis

GC-MS was performed with an Agilent 6890 GC instrument coupled to an Agilent 5973 mass spectrometer and an Agilent ChemStation software (Agilent Technologies). A HP-5MS capillary column (30 m x 0.25 mm i.d.) coated with 0.25 μm film of 5% phenyl methyl siloxane was used for separation. The extracted ion chromatogram (EIC) (Chromatogram created by plotting the intensity of the signal observed at a chosen m/z value) of GC–MS was applied for accurate quantification of camphor, borneol and bornyl acetate. Characteristic fragment ions, m/z 95 was selected for the GC–MS quantification (Lv et al., 2015).

Field investigation

To collect data about technology challenges, ecological impacts and economic benefits, field investigation was designed and carried out in 2014. First, data was collected from exploratory interviews (n = 2) with government staff of Yangchun Municipal Finance Bureau and experts of Yangchun Agricultural Bureau. These interviews helped to collect data of the financial, commercial and regulatory situation affecting A. Villosum development. Second, the field interviews (n = 10) were also conducted with administrators, farmers and interns (students) of three A. Villosum cultivation bases in Yangchun area. These interviews provided a significant understanding of the current situation of different production modes of A. Villosum. Third, the information of natural environment of these three production modes was collected on site. The summary of field investigation was shown in Table 2.
Table 2: Summary of field investigation

| Data sources          | Data                                                                 |
|-----------------------|----------------------------------------------------------------------|
| Government staff      | Financial, commercial and regulatory situation affecting *A. Villosum* development |
| Farmers               | Technical requirements (planting, collecting), cost-effectiveness (cost structure, input, output), ecology impact (positive, negative) |
| Interns (students)    | Technical requirements (planting, collecting), cost-effectiveness (cost structure, input, output), ecology impact (positive, negative) |
| Base administrators    | Technical requirements, input and output                              |
| Expert                | Opinions of three production modes                                   |

**Results**

**Current situation of three production modes in Yangchun**

For wild collecting, by the end of 2013, total area of wild *A. Villosum* was estimated to be 0.41 km², mainly distributed in intermountain valley of Panlong Village (Figure 1-a). For artificial cultivating, total area was about 42 km², mainly distributed in Kongdong Village, Jiuzikeng Village, Heshuinaruan Village, and Yongningshuangdi Village of Chunwan District (Figure 1-b). For natural fostering, total area was about 15.33 km², and mainly distributed in the middle of the valley and the two sides of mountain pit, where Panlong is a well-known location. Natural fostering was mostly operated by local farmers (see Figure 1-c).

Herbal quality analysis

The samples were prepared and subsequently determined by GC-MS. The contents of other compounds (Copaene, Caryophyllly + α-Santalene, γ-Elemene, δ-Cadinol, δ-Cadinene, Torreyol, Methenolone) were estimated approximately by using calibration curve of borneol, which is one of the major components in *A. Villosum*. Specifically, the chromatogram of *A. Villosum* (sample S9) is shown in Figure 2. In addition, based on the GC-MS total ion chromatograms of 12 samples, the main components of all the samples were the same, including camphor, borneol, and bornyl acetate. Supplementary information of GC-MS total ion chromatograms of the other 11 samples is available in the attachment.
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Figure 2: GC-MS total ion chromatograms of A. Villosum (Sample S9)
(1. Camphor, 2. Borneol, 3. Bornyl acetate, 4. Copaene, 5. Caryophyll + α-Santalene, 6. γ-Elemene , 7. δ-Cadinol, 8. δ-Cadinene, 9. Torreyol, 10. Methenolone)

For identifying the components in A. Villosum samples, the MS matching results of the main components in A. Villosum showed that camphor, borneol and bornyl acetate are the main components (Table 3).

### Table 3: MS matching results of the main components in A. Villosum (total samples)

| Retention Time (Peak no.) | Compound             | MW  | EIC<sup>a</sup> | Molecular Formula |
|--------------------------|----------------------|-----|-----------------|-------------------|
| 9.885 (1)                | Camphor              | 152.23 | 95             | C<sub>10</sub>H<sub>16</sub>O |
| 10.361 (2)               | Borneol              | 154.25 | 95             | C<sub>10</sub>H<sub>18</sub>O |
| 13.099 (3)               | Bornyl acetate       | 196.29 | 95             | C<sub>12</sub>H<sub>20</sub>O<sub>2</sub> |
| 15.114 (4)               | Copaene              | 204.35 | -              | C<sub>15</sub>H<sub>24</sub> |
| 16.778 (5)               | Caryophyll + α-Santalene |   | -              | C<sub>15</sub>H<sub>24</sub> |
| 19.293 (6)               | γ-Elemene            | 204.35 | -              | C<sub>15</sub>H<sub>24</sub> |
| 19.940 (7)               | δ-Cadinol            | 204.35 | -              | C<sub>15</sub>H<sub>24</sub> |
| 20.204 (8)               | δ-Cadinene           | 204.35 | -              | C<sub>15</sub>H<sub>24</sub> |
| 22.170 (9)               | Torreyol             | 222.37 | -              | C<sub>13</sub>H<sub>24</sub> |
| 32.607 (10)              | Methenolone          | 302.45 | -              | C<sub>22</sub>H<sub>22</sub>O<sub>3</sub> |

Note: a, Ions for extracted ion chromatograms.; “—”, not analyzed in EIC mode.

Table 4 further illustrated the comparison of the contents of main components in different samples. Natural fostering from Chunwan (S8) has the highest content in camphor (3.47 mg/g) and borneol (1.63 mg/g). For bornyl acetate, artificial cultivating from Yongning (S12) has the highest content (20.88 mg/g).

For the total contents of the three main components, among all the samples, S12 (23.5 mg/g) has the highest contents. However, the average contents of the three main components in wild collecting (S1, S2, S3) was 13.44 mg/g, natural fostering (S4, S5, S6, S7, S8) was 18.76 mg/g, and artificial cultivating (S9, S10, S11, S12) was 18.64 mg/g. Therefore, natural fostering had higher content of main components than wild collecting and artificial cultivating.

In addition, in Heshui, the samples of natural fostering have higher contents than artificial cultivating; in Panlong, the samples of natural fostering have higher contents than wild collecting; but in Chunwan, there are no obvious difference between natural fostering and artificial cultivating.

### Table 4: Contents (mg/g) of main components in different A. Villosum (total samples)

| Compounds                     | Samples |
|-------------------------------|---------|
|                              | S1  | S2  | S3  | S4  | S5  | S6  | S7  | S8  | S9  | S10 | S11 | S12 |
| Camphor                       | 2.20 | 0.71 | 0.74 | 2.05 | 3.43 | 2.82 | 1.93 | 3.47 | 1.12 | 1.42 | 2.31 | 1.33 |
| Borneol                       | 0.44 | 0.26 | 0.32 | 1.47 | 0.56 | 0.41 | 1.59 | 1.63 | 1.05 | 0.70 | 0.40 | 1.29 |
| Bornyl acetate                | 12.00 | 10.4 | 13.1 | 12.2 | 14.9 | 14.0 | 15.9 | 17.3 | 17.7 | 13.3 | 12.9 | **20.8** |
| Copaene                       | 0.10 | 0.06 | 0.23 | 0.24 | 0.19 | 0.15 | 0.27 | 0.35 | 0.33 | 0.28 | 0.24 | 0.42 |
| Caryophyll + α-Santalene      | 0.13 | 0.06 | 0.15 | 0.17 | 0.13 | 0.13 | 0.19 | 0.22 | 0.22 | 0.17 | 0.15 | 0.29 |
| γ-Elemene                     | 0.25 | 0.19 | 0.41 | 0.21 | 0.33 | 0.31 | 0.19 | 0.15 | 0.19 | 0.13 | 0.26 | 0.28 |
| δ-Cadinol                     | 0.09 | 0.04 | 0.06 | 0.06 | 0.05 | 0.19 | 0.06 | 0.07 | 0.08 | 0.05 | 0.04 | 0.09 |
| δ-Cadinene                    | 0.05 | 0.03 | 0.06 | 0.06 | 0.06 | 0.06 | 0.07 | 0.08 | 0.08 | 0.06 | 0.06 | 0.09 |
| Torreyol                      | -    | 0.05 | 0.02 | 0.02 | 0.04 | 0.03 | 0.06 | 0.08 | 0.07 | -    | 0.06 | 0.07 |
| Methenolone                   | 0.46 | -    | 0.04 | 0.17 | 0.13 | 0.05 | 0.07 | 0.36 | 0.29 | 0.36 | 0.36 | 0.38 |

Note: “—”, Undetected
By comparing the average contents of all the components of natural fostering, artificial cultivating and wild collecting samples, it showed that the difference between natural fostering and artificial cultivation *A. Villosum* is not obvious, but both of them are better than the wild collecting (Figure 3).

![Figure 3: Comparison of the average contents of the main components of A. Villosum in wild collecting, natural fostering and artificial cultivating](image)

**Production technology challenges**

Table 5 provides a comparative summary of the technological challenges of the three production modes. For the investigation of technology challenges, there are no technical requirements at all in the production mode of wild collecting. It is very different in the natural fostering because many planting technologies are required, including seed selection, seeding, artificial pollination, fertilization and pest control. However, in the artificial cultivating, most attention should be paid on location selecting, artificial pollination, and scientific harvesting.

| Wild collecting | Artificial cultivating | Natural fostering |
|-----------------|-----------------------|-------------------|
| • None          | • location selecting  | • seed selection  |
|                 | • artificial pollination | • seeding       |
|                 | • scientific harvesting | • artificial pollination, fertilization |
|                 |                       | • pest control    |

**Ecological impacts**

Regarding ecological impact analysis of wild collecting and natural fostering of *A. Villosum*, both production modes were good for protecting ecological environment. Particularly, wild collecting was very helpful for protection of biodiversity and germplasm resources. Artificial cultivating can improve water and soil loss, enhance plant population and improve the orchard environment, but pesticide residues and heavy metal pollution cannot be ignored (Table 6).

| Wild collecting | Artificial cultivating | Natural fostering |
|-----------------|-----------------------|-------------------|
| • good for protecting the biodiversity and germplasm resources | • improve water and soil loss | • good for protecting ecological environment |
|                 | • enhance plant population |                       |
|                 | • improve the orchard environment |                       |
|                 | • but cause pesticide residues and heavy metal pollution |                       |

**Economic benefits**

Economic input and output analysis was showed as Table 7. The economic input and output analysis is calculated in one-year period of one km$^2$ area. It is obvious that *A. Villosum* in wild collecting had the highest price while *A. Villosum* in artificial cultivating had the lowest price. However, the highest turnover was natural fostering in Panlong with 14,080 RMB per km$^2$. It should be noticed that the price of natural fostering in Panlong was much higher than other areas because of brand effect. In terms of cost, the cost structure of different patterns was different, but labor cost was the main cost of all the patterns. The total cost of artificial
Economic input and output comparison of three production modes

|                      | Wild collecting | Artificial cultivating | Natural fostering (Panlong) | Natural fostering (other areas except Panlong) |
|----------------------|-----------------|-----------------------|----------------------------|-----------------------------------------------|
| Fresh fruit yield (Kg) | 2               | 40                    | 22                         | 22                                            |
| Price (RMB per Kg)    | 1,000           | 180                   | 640                        | 240                                           |
| Turnover (RMB)        | 2,000           | 7,200                 | 14,080                     | 5,280                                         |
| Labor cost (RMB)      | 90              | 1,800                 | 1,600                      | 1,200                                         |
| Land rent (RMB)       | 0               | 200                   | 0                          | 0                                             |
| Seedling (RMB)        | 0               | 960                   | 0                          | 0                                             |
| Fertilizer (RMB)      | 0               | 500                   | 200                        | 200                                           |
| Pesticide (RMB)       | 0               | 100                   | 0                          | 0                                             |
| Total cost (RMB)      | 90              | 3,560 (2,600)         | 1,800                      | 1,400                                         |
| Gross profit (RMB)    | 1,910           | 3,640 (4,460)         | 12,280                     | 3,880                                         |
| Profit margin (%)     | 95.5            | 50.6 (63.9)           | 87.2                       | 73.5                                          |

*a the comparison in per km² per year; b the total cost per year without seedling; c the gross profit per year without seedling; d the profit margin per year without seedling

Discussion

Comparison of the main components of A. Villosum of three production modes

The chemical components of essential oil among A. Villosum are important quality indicators (Wang et al., 2013). Researchers found that the principal components of volatile oils were similar (Wang et al., 2013; Zhang et al., 2011; Yan et al., 2014). However, the percentages of each component in these volatile oils were different (Fu et al., 2010). Our study found that A. Villosum from natural fostering has higher average contents of the main components.

Natural fostering is a production mode that combines wild and artificial cultivation, which brings several advantages for planting A. Villosum. Firstly, in the process of seed selection, only the high-class germplasm resource is selected for natural fostering. Secondly, natural fostering is mainly operated by local farmers who have much experience of planting A. Villosum. Particularly, the artificial pollination can improve the efficiency of fructification. Finally, the location selection is very important for planting A. Villosum. The ideal location should meet these conditions: the altitude should be lower than 500 meters; the slope should be less than 30 degrees; the shade should be among 50% and 60%; and it must be planted in loam with yellow soil as subsoil and loose topsoil. In addition, the most important condition is that it must grow under trees. All these conditions specify the growing environment for A. Villosum. Therefore, natural fostering with the optimized combination of these environmental requirements can produce A. Villosum with high content.

Comparison of technology challenges, enological impact and economic benefits of three production modes

As shown in this study, different production modes have different development advantages. It is obvious that they are very different in production scale. The scale of wild A. Villosum is very small, while the emerging production mode of artificial cultivating is rapidly increasing. However, the scale only reflects the current situation of different production modes. The factors of technological challenges, ecological impact and economic benefits need to be considered.

For technological challenges, artificial cultivating of A. Villosum needs to consider location selecting, artificial pollination and scientific harvesting. In particular, the artificial pollination was especially the key of high yield, which still has many technological problems unsolved. In addition, how to identify and protect the high-class germplasm resources is another technological challenge.

For ecological impacts, both wild collecting and natural fostering are good for protecting ecological environment. On the contrary, while artificial cultivating can improve water and soil loss, enhance plant population and improve the orchard environment, it leads to pesticide residues and heavy metal pollution. The cultivation of A. Villosum in Xishuangbanna area of Yunnan Province had significantly reduced plant diversity, tree biomass, litter production and soil nutrients, which would affect the structure and function of the seasonal rain forest (Liu et al., 2006).

Moreover, economic benefits play a fundamental role for planting A. Villosum. As shown in this study, while the price of wild collection is the highest, it is constrained by quantity. Considering the final profit, natural fostering is better than the other two modes. Hence, based on comprehensive consideration of technological challenges, ecological impact and economic benefits, natural fostering shows its advantages over wild collecting and artificial cultivating.

The medical efficacies of A. Villosum of different production modes

As different production modes and ecological habitats can result in varied plant morphology and medical traits (Feng et al., 2004; Guo & Liu, 2009; Zhang et al., 2005; Zhang, 2008), it is more important to consider pharmacological effects of A. Villosum. There
have been studies that focused on medical application of A. villosum (Jafri et al., 2001; Mathew et al., 2003; Zhao et al., 2009; Zhao et al., 2011; Lee et al., 2012; Zhang & Shen, 2013). However, there are fewer studies on medical efficacies of A. villosum from different production modes (Ding et al., 2004; Liu et al., 2013; Peng et al., 2006).

For chemical components of A. villosum, previous studies identified the main components of A. villosum as camphor, borneol and bornyl acetate (Chen et al., 2014; Yin et al., 2008; Wang & Situ, 2010; Zhang et al., 2011), which is similar with the results of our GC-MS analysis. However, our study found that natural fostering generates higher content of these main components. Therefore, it is expected that A. villosum of natural fostering can have more significant efficacies, which is worth deeper clinical exploration.

Implications and future study

Through comprehensive comparison of three production modes, this study has several implications for sustainable development of A. villosum. Firstly, among the three production modes, natural fostering is more recommended while the other two production modes can play complementary roles in some aspects. Secondly, location selecting should be emphasized as natural fostering of A. villosum is very sensitive to location environment. Thirdly, organic fertilizer is most suitable for natural fostering. Fourthly, scientific artificial pollination is crucial for both natural fostering and artificial cultivating.

In addition, some research limitations can be addressed through future studies. Firstly, in this study, we only chose Yangchun A. villosum as the analysis sample. Future study could extend to test samples from other areas like Hainan Province, Yunnan Province, Guangxi Province, and Laos. greatly helpful to the development of sustainable production modes of A. villosum. Secondly, future study can apply more technical methods to measure environmental factors and test their impacts on A. villosum in different production mode. It will contribute to establish a systematic model of understanding environmental factors on sustainable production modes of A. villosum.

Conclusion

This study found that the wild A. villosum had low output and was in an endangered situation. The A. villosum of artificial cultivation had larger agriculturing area with higher productivity, but faced ecological challenges. Lastly, the A. villosum from natural fostering generated the highest economic benefit and relatively low ecological impact. In addition, the natural fostering type had relatively better quality than the other types. Therefore, natural fostering is suggested for sustainable development of A. villosum.

Conflict of interest: The authors declare that there is no conflict of interests regarding the publication of this paper.

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