

The invasive alien species *Amorpha fruticosa* in Bulgaria and its potential as economically prospective source of valuable essential oil

Ekaterina Kozuharova¹, Niko Benbassat¹, Iliana Ionkova¹

¹ Medical University of Sofia, Department of Pharmacognosy, Faculty of Pharmacy

Corresponding author: Ekaterina Kozuharova (ina_kozuharova@yahoo.co.uk)

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Abstract

The high tolerance of various habitat conditions and potent propagation ability of *Amorpha fruticosa* L. (Fabaceae) promote its aggressive invasive behaviour. The aim of this study is to evaluate 1) several populations of this plant by the potential yield of the fruit, 2) the approximate yield of the essential oils, and 3) composition of the essential oil.

The potential yield of fruit is evaluated based on extrapolations of weight and number of fruits per infructescence, number of infructescences per plant and number of individuals per population. Steam extraction of the essential oil was performed and GS/MS analysis of the composition of the essential oil.

The populations on our key plots are big enough for harvesting with fruit/infructescence 152 ± 15 ($n = 20$) and infructescences/shrub 436 ± 157 ($n = 20$). The yield is 0.83 ml/100g. We identified 22 components with major constituents caryophyllene (17.64%) α -guaiene (14.70%), naphthalene (6.75%), γ -muurolene + (5.98%).

Keywords

Amorpha fruticosa, chemical composition, essential oil, yield

Introduction

Indigo bush, *Amorpha fruticosa* L. (family Fabaceae) is a 1–3 m tall shrub with odd-pinnate compound leaves with stipules and 9–35 ovate or elliptical, entire leaflets. The purple flowers are clustered in racemes. The fruit is an indehiscent pod of 8–9 mm. The plant is native to North America, and it is widely distributed in the US, southern Canada and northern Mexico (Wilbur 1975; USDA NRCS 2009; USDA, ARSNPGS 2019). *A. fruticosa* was introduced in Europe as an ornamental, honey and protective against erosion plant (Petrova et al. 2012; Kozuharova et al. 2017; CABI 2019) but turned into aggressive invasive

species and now is included in the list of “Worst invasive alien species threatening biodiversity in Europe” (Petrova et al. 2012; Monaco 2014; CABI 2019). *A. fruticosa* is widely distributed in Bulgaria along roadsides where it is often planted, and it forms large monodominant, dense groups along rivers and reservoir banks, replacing native species and altering the structure of native plant communities (Petrova et al. 2012; Zahariev 2014). *A. fruticosa* not only overcompete the local plants. It contains compounds that suppress their seed germination and seedling developments (Pavićević 2013; Hovanet et al. 2015; Martinović 2018) and its juglon index is 1.11–2.00 depending on extract concentrations (Csiszár 2009). Typically for a leguminous

plant, *A. fruticosa* contains a set of family marker classes such as rotenoids (Kozuharova et al. 2018). Namely, the rotenoid compounds (e.g. amorfigenin, rotenone etc.) are known to have phytotoxicity (Simin et al. 2002). Additionally *A. fruticosa* is attacked only by several more or less specialized insects (Petrova et al. 2012) because the plant contains compounds with insecticide activity (Brett 1946). These compounds are the rotenoids (Moring and McChe-sney 1979; Berenbaum 1989; Kole et al. 1992; Konoshima et al. 1993; Fang and Casida 1998; Ivanescu et al. 2014; Kariuki et al. 2014; Mingshan et al. 2015). In fact rotenone is a botanical insecticide, which has been used for centuries and is still used worldwide (Gupta 2007).

A. fruticosa is difficult to control as it propagates by seeds, which are produced in large quantities and have high germination rate. Additionally there is considerable vegetative propagation. The seeds are driven by the water to the moist places, which the plant prefers but it also tolerates both prolonged droughts and prolonged flooding, as well as wide range of light and soil conditions including salinity (Petrova et al. 2012; Ciuvat et al. 2016).

Amorpha fruticosa contains number of bioactive compounds with valuable pharmacological effects such as antimicrobial, wound healing, hepatoprotective and osteoclast inhibitory effects, anticancer properties etc., and its potential against diabetes and metabolic disease is rather high

(Kozuharova et al. 2017). The essential oil of *A. fruticosa* fruits varies qualitatively and quantitatively depending on the maturity stage, drying process and storage, as well as location of the plant populations/ecological factors (Georgiev et al. 2000; Lis and Góra 2001; Lis et al. 2001; Stoyanova et al. 2003; Ivanescu et al. 2014; Chen et al. 2017). Flowers and leaves also produce essential oil with different composition compared to the fruits (Lis and Góra 2001).

The aim of this study is to evaluate 1) the potential fruit yield of several populations of *Amorpha fruticosa*, 2) the approximate yield of the essential oil, 3) the composition of the essential oil from Pasarel locality.

Material and methods

Amorpha fruticosa resources estimation

In order to evaluate exploitation potential of *Amorpha fruticosa* we selected key plots at four localities (harvesting areas) in Bulgaria. These are as follows: 1) the banks and adjusted area of Ivailovgrad reservoir, 2) the banks and adjusted area of Koprinka reservoir, 3) the banks of Struma river between the towns of Blagoevgrad and Simitly, 4) the banks and adjusted area of Iskar river above village Pasarel, 5) The roadside in the vicinity of Djulino village (Table 1;

Table 1. Estimated resources of *Amorpha fruticosa* at four key plots in Bulgaria.

| Locality | Area of the yield gathering territory [ha] | Projective cover of <i>A. fruticosa</i> in the key plot [%] | Area taken by <i>A. fruticosa</i> in the key plot [ha] | Fruit/infrutescence; infrutescences/shrub | Average resource of the key plot [kg] |
|-----------------------|--|---|--|---|---------------------------------------|
| Ivailovgrad reservoir | ~ 10 | 95 | 0.00005 | 152 ± 35 (n = 20); 436 ± 157 (n = 20) | 612.5 |
| Koprinka reservoir | ~ 4 | 90 | 0.00003 | 165 ± 24 (n = 20); 440 ± 149 (n = 20) | 375.0 |
| Struma river | ~ 15 | 94 | 0.00006 | 148 ± 35 (n = 20); 449 ± 150 (n = 20) | 800.0 |
| Village Pasarel | ~ 1 | 91 | 0.00001 | 155 ± 37 (n = 20); 420 ± 160 (n = 20) | 62.5 |
| Village Djulino | ~ 4 | 92 | 0.00003 | 142 ± 34 (n = 20); 415 ± 152 (n = 20) | 187.4 |

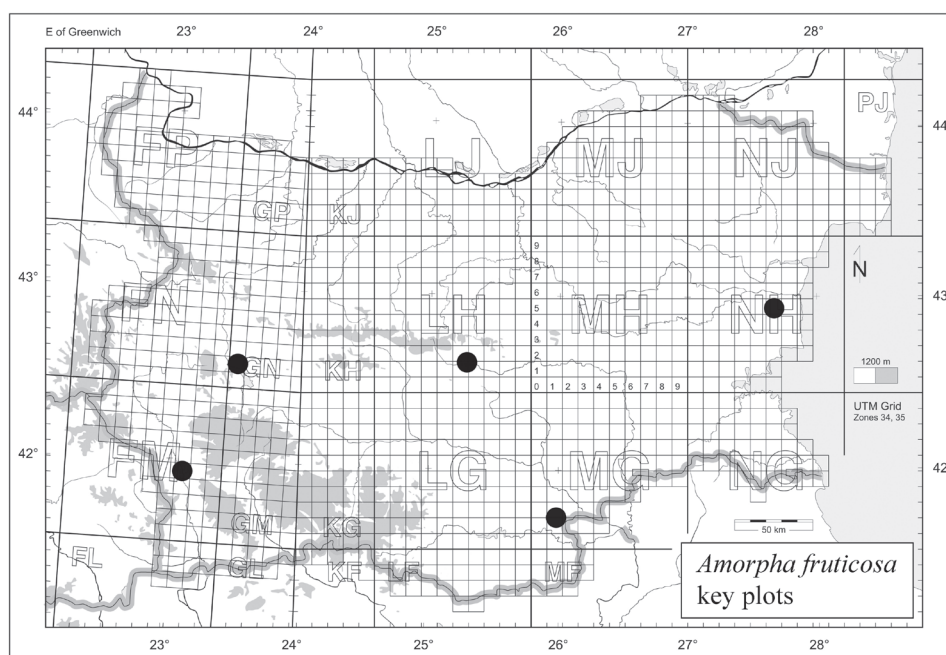


Figure 1. Key plots.

Fig. 1). We followed the “Methodology for assessment of medicinal plant resources” developed by Shroeter and co-authors (Shroeter et al. 1986) modified and adapted to the specifics of this invasive species. For *A. fruticosa* fruit yield assessment we chose the method of “key plots”. We did transects within each key plot with 1–10 measuring plots of a size 10×10 m along transects in accordance to the “key plot” area. The “key plot” approach is used for plant species specialized to particular habitats. According to this methodological approach we determined the area where individuals of *A. fruticosa* are distributed within each key plot as a projective cover [%] (Table 1). We used GPS and “Garmin Etrex Vista CX” receiver to measure the total area of the gathering territory, the area of each key plot, and based on that we calculated the area occupied by the individuals of *A. fruticosa* (Table 1). The potential expected yield of fruit (kg/ha) is evaluated based on extrapolations of number of fruits per infrutescence, number of infrutescences per plant and number of individuals per population, calculated by the projective cover of a shrub. The weight of 10 samples of 100 fruits each was measured and the average was calculated. Based on the average fruit set per plant, number of individuals and their weight the extrapolations were performed. Additionally we double checked the expected resources of fruit for each of the key plots by measuring the weight of entire fruit yield of a shrub with cover of 4 m².

Essential oil extraction and analysis

Amorpha fruticosa fruit was collected at Passarel locality in third decade of October 2018. It was kept at room temperature until the extraction in May 2019. Steam extraction of the essential oil was performed using Clevenger apparatus for 4h. GS/MS analysis of the composition of the essential oil was performed. Gas chromatography-mass spectroscopy (GC/MS) analysis was conducted according to Ph. Eur. 9th. The GC-MS analysis of diluted (1:1000) *A. fruticosa* essential oils was performed on Exactive Orbitrap GC-MS system (ThermoFisher Scientific) operating at 70 eV, ion source temperature 230 °C, interface temperature 280 °C with split injection (1 µL, ratio 20:1) at 270 °C injector temperature. A fused silica capillary column, 5% phenyl/95% methyl polysiloxane (TG-5SILMS 30 m × 0.25 mm × 0.25 µm, Thermo) was used.

Results and discussion

Amorpha fruticosa resources

The extrapolation of the data reveals that: *Amorpha fruticosa* forms dense patches (with >90% cover) in shape of belts following the banks (Table 1; Fig. 2). There are hundreds of inflorescence/infrutescences (Fig. 2). The propagation is basically by seed. Numerous small seedlings were observed in the key plots. Also seedlings were detected to appear immediately after the water level dropped and opened the soil, as the bottom of reservoir Ivailovgrad was completely dry



Figure 2. Infrutescences of *Amorpha fruticosa* at early maturing stage. Shrubs at Ivaylovgrad reservoir bank on 14th of August 2019.



Figure 3. The bottom of reservoir Ivailovgrad drained for less than three months, spackled with grass and seedlings of *Amorpha fruticosa*; The banks densely covered with a belt of adult, generative *A. fruticosa* shrubs.

in August 2019 (Fig. 3). *A. fruticosa* over competes the local vegetation on the banks (Fig. 2). The high local resource of *Amorpha fruticosa* is due to the lavish fruit set, numerous fruits per infrutescences and numerous infrutescences per plant as well as the dense cover of this plant in the localities. The populations on our key plots are big enough for harvesting (Table 1; Figs 2, 4) with average fruit/infrutescence 155 (n = 100) and infrutescences/shrub 432 (n = 100).



Figure 4. Infructescence and fruits of *Amorpha fruticosa*.

Essential oil

The essential oil of *Amorpha fruticosa* basically is not difficult to extract. The fruit surface is more or less heavily beset with conspicuous pustulate, resinous glands (Fig. 4).

The yield is 0.83 ml/100g. We identified 22 components (Table 2; Fig. 5) with major constituents: caryophyllene (17.64%) α -guaiene (14.70%), naphthalene (6.75%), γ -muurolene + (5.98%).

A considerable qualitative and quantitative composition variability of the *Amorpha fruticosa* essential oil is observed (Table 3). The yield varies between 0.32% and 1.50% and depends on the locality, possibly the extraction method and definitely the ripe fruit produces more essential oil (Table 3). Only α -pinene, myrcene, caryophyllene and γ -muurolene + are universal components for all *A. fruticosa* essential oil samples but even they were presented in different quantitative values (Table 3).

Conclusion

The essential oil of *Amorpha fruticosa* fruit varies qualitatively and quantitatively depending on the maturity stage, drying process and storage, as well as locality of the plant populations/ecological factors (Table 3) and

Table 2. Chemical composition (%) of essential oil from *Amorpha fruticosa* fruit collected at Passarel locality in third decade of October 2018.

| | Major constituents | Rt (min) | Exat Mass | Formula | % |
|----|---|----------|------------|-----------------------------------|-------|
| 1 | 3-Carene | 6.73 | 136.1252 | C ₁₀ H ₁₆ | 4.17 |
| 2 | β -Pinene | 8.50 | 136.1252 | C ₁₀ H ₁₆ | — |
| 3 | trans- β -Ocimene | 9.96 | 136.1252 | C ₁₀ H ₁₆ | 1.98 |
| 4 | alfa.-Copaene | 10.28 | 204.1878 | C ₁₅ H ₂₄ | — |
| 5 | Ylangene | 18.74 | 204.1878 | C ₁₅ H ₂₄ | 1.81 |
| 6 | γ -Muurolene | 18.89 | 204.1878 | C ₁₅ H ₂₄ | 5.98 |
| 7 | Caryophyllene | 19.91 | 204.1878 | C ₁₅ H ₂₄ | 17.64 |
| 8 | Aromandendrene | 20.33 | 204.1878 | C ₁₅ H ₂₄ | 1.11 |
| 9 | cis- β -Farnesene | 20.60 | 204.1878 | C ₁₅ H ₂₄ | 2.81 |
| 10 | Humulene | 20.73 | 204.1878 | C ₁₅ H ₂₄ | 2.49 |
| 11 | β -Muurolene | 21.13 | 204.1878 | C ₁₅ H ₂₄ | 2.49 |
| 12 | Benzene, 1-(1,5-dimethyl-4-hexenyl)-4-methyl- | 21.24 | 202.172151 | C ₁₅ H ₂₂ | 4.23 |
| 13 | α -Guaiene | 21.56 | 204.1878 | C ₁₅ H ₂₄ | 14.70 |
| 14 | Guaia-1(10,11-diene)]- | 21.74 | 204.1878 | C ₁₅ H ₂₂ | 0.67 |
| 15 | R)-1-Methyl-4-(6-methylhept-5-en-2-yl)cyclohexa-1,4-diene | 21.87 | 204.1878 | C ₁₅ H ₂₂ | 2.15 |
| 16 | Naphthalene, 1,2,3,4,4a,5,6,8a-octahydro-7-methyl-4-methylene-1-(1-methylethyl)-, (1 α ,4 α ,8 α)- | 21.97 | 204.1878 | C ₁₅ H ₂₂ | 6.75 |
| 17 | 1-Isopropyl-4,7-dimethyl-1,2,3,5,6,8a-hexahydronaphthalene | 22.09 | 204.1878 | C ₁₅ H ₂₂ | 11.00 |
| 18 | β -Longipinene | 22.18 | 204.1878 | C ₁₅ H ₂₂ | 1.78 |
| 19 | Cubenene | 22.32 | 204.1878 | C ₁₅ H ₂₂ | 1.31 |
| 20 | α -Calacorene | 22.56 | 200.1565 | C ₁₅ H ₂₀ | 0.63 |
| 21 | Caryophyllene oxide | 23.46 | 220.1727 | C ₁₅ H ₂₄ O | 1.11 |
| 22 | 2-Naphthalenemethanol, 1,2,3,4,4a,5,6,8a-octahydro- α , α ,4a,8-tetramethyl-, [2R-(2 α ,4 α ,8 α)]- | 25.25 | 222.1984 | C ₁₅ H ₂₆ O | 1.69 |

Table 3. Comparison of the major constituents of *Amorpha fruticosa* fruit essential oil detected by various researchers.

| Major constituents | Our results | Georgiev et al. 2000 | Lis et al. 2001 | Lis et al. 2001 | Lis and Góra 2001 | Ivanescu et al. 2014 |
|--|----------------------------|-------------------------|---------------------|-------------------|----------------------------|---|
| | Bulgaria 18 ripe fruit [%] | Formation to ripe fruit | Bulgaria ripe fruit | Poland ripe fruit | Unripe to dried ripe fruit | Romania Loc. S2 October 2011 ripe fruit [%] |
| yeald | 0.86 | 0.32–0.72 | 0.60 | 1.40 | 0.45–1.36 | 1.50 |
| α -pinene | 10.86 | 1.20–4.10 | 4.90 | 19.55 | 25.80–19.60 | 10.86 |
| myrcene | 1.38 | 0.80–1.60 | 1.59 | 17.90 | 13.30–18.70 | 1.38 |
| ar-curcumene | — | 12.20–18.10 | — | — | 4.80–6.00 | — |
| β -caryophyllene | — | 5.20–11.50 | 5.20 | 4.08 | 2.30–4.10 | 3.20 |
| caryophyllene | 17.64 | — | — | — | — | — |
| γ -muurolene + | 5.98 | 13.20–18.10 | 13.10 | 4.50 | 4.30–5.30 | 7.30 |
| γ -cadinene | — | 7.90–10.70 | 9.53 | 3.41 | 2.10–2.80 | 3.19 |
| δ -cadinene | — | 14.40–17.30 | 14.93 | 6.89 | 5.10–6.90 | 5.82 |
| α -guaiene | 14.70 | — | — | — | — | — |
| naphthalene | 6.75 | — | — | — | — | — |
| 1-Isopropyl-4,7-dimethyl-1,2,3,5,6,8a-hexahydronaphthalene | 11.00 | — | — | — | — | — |

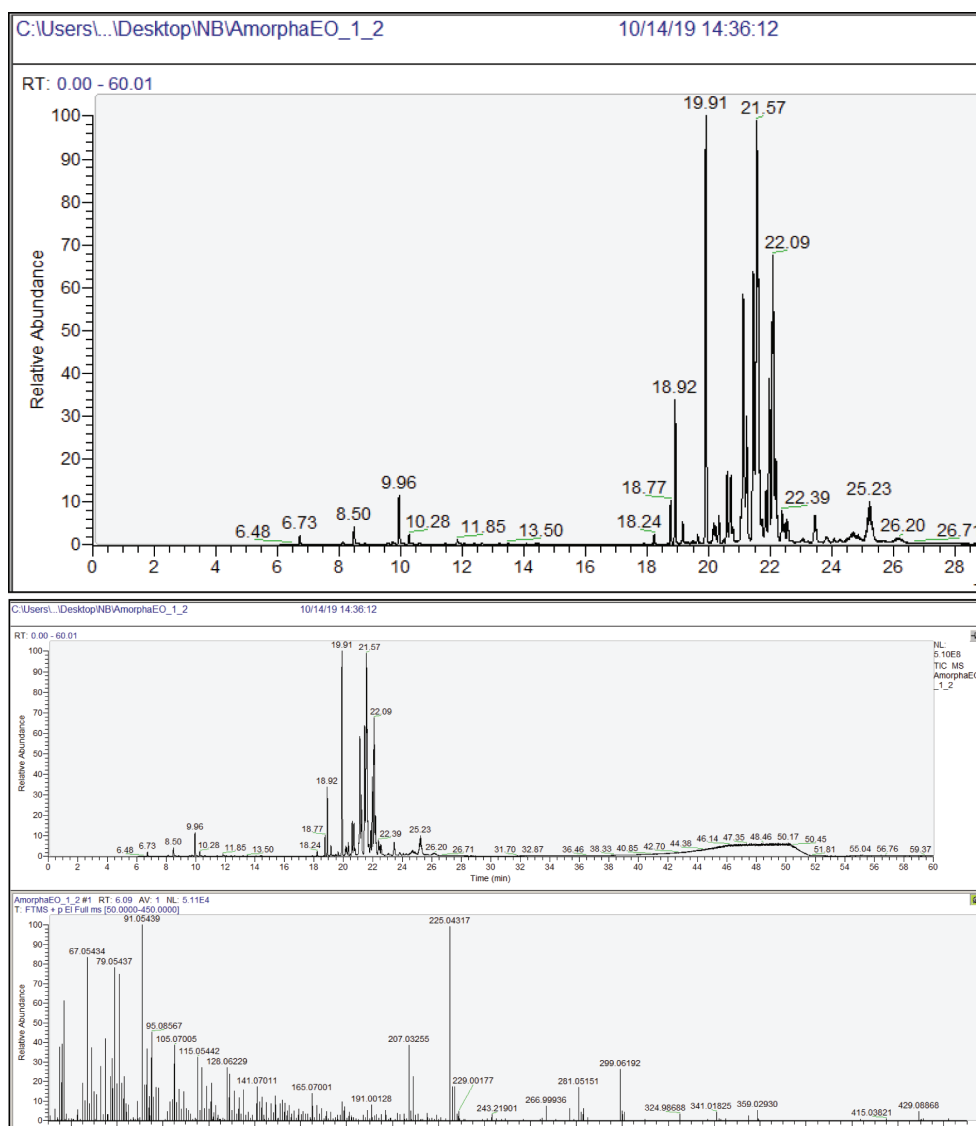


Figure 5. GS/MS analysis of the composition of *A. fruticosa* essential oil.

our results confirm the statement of other researchers (Georgiev et al. 2000; Lis and Góra 2001; Lis et al. 2001; Stoyanova et al. 2003; Ivanescu et al. 2014; Chen et al. 2017). The intensive balsamic, long lasting odour the oil can be used in perfumery. Also it possesses antimicrobial and wound healing activity. (Das et al. 2007; Qu et al. 2013; Ivanescu et al. 2014). The high content of δ -cadinene in some origins (Table 3, Lis et al. 2001) suggests fungant and repellent effects (Licciardello et al. 2013). Fumgant and repellent effects possess also α -pinene (Angelini et al. 2003; Wright et al. 2013; Polatoğlu et al. 2013) which is presented in all samples (Table 3). The high content of caryophyllene/ β -caryophyllene (Table 3)

suggest anti-inflammatory and local anesthetic activity (Ghelardini et al. 2001; Gertsch et al. 2008). It also can be efficient for biological pest control as it is attractant for the green lacewing (Flint et al. 1997), a well known beneficial insects, as with lady beetles, these natural enemies are important predators.

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