## File 0 - Plants : introduction



First and foremost, it should be noted that the term « Artemisia » often used by La Maison de l'Artemisia refers to the plant species Artemisia afra and Artemisia annua. This generic term is not written in italics so as not to confuse it with the genus « Artemisia » which comprises several hundred other species.

#### Distinction between Artemisia annua and Artemisia afra :

*Artemisia annua* is an herbaceous plant that has been used for 2000 years in Traditional Chinese Medicine to prevent and treat intermittent fevers (malaria) and other parasitic diseases. It is an annual pant and must therefore be sown every year in order to be harvested before flowering. This makes it demanding in terms of care.

Artemisia afra is a perennial bush native to South East Africa, used by Traditional Medicine practitioners for centuries to prevent and cure malaria and other parasitic diseases. It is a perennial plant which can be harvested as needed throughout its growth. However, it is difficult to produce viable seeds. This is why it is mainly propagated by layering or cuttings.



Figure 1 : Artemisia afra bush (bottom left), flowering Artemisia annua plant with small yellow blossoms (centre right) and Artemisia annua plants (extreme right and in the background).

## Artemisia annua



#### 1. Taxonomy

Artemisia annua L. is a species of the Asteraceae family.

It has many local names including sweet wormwood, annual wormwood, sweet Annie, sweet sagewort, annual mugwort in English; armoise annuelle, absinthe chinoise in French and mohlaswapatla in South Africa [1-2]

Its Chinese name is qinghao (青蒿) [3].

#### 2. Origin and distribution

Artemisia annua is a plant native to the high plateaux of China, where it grows in steppe vegetation (40° north latitude between 1000 and 1500m altitude). It has spread widely throughout the world: North China, Europe, North Africa, North India, North Vietnam, USA, Argentina ...

Over the last thirty years, it has been introduced in East Africa and Madagascar to establish large plantations (Kenya, Madagascar, Ethiopia, Tanzania ...), in Central Africa (Burundi, Cameroon, DRC, Rwanda, Sudan, Uganda ...), in West Africa (Burkina, Gambia, Mali, Nigeria, Senegal, Togo ...) and in South America (Brazil, Peru, Colombia) in a more marginal way. [1,3,4]

#### 3. Botanical description

The morphology of Artemisia annua varies enormously from one plant to another.

- An annual herbaceous plant forming bushes that can exceed 3 metres in height. Potentially biennial [1,4,6].
- Root system consisting of a short taproot and numerous secondary roots [4].

- Usually composed of a very hard single upright fibrous stem, occasionally several several stems, with alternating branches that can reach a level higher than n+4 (quaternary branches) [3,4].
- Various ports are possible depending on the type of branching (slender, pyramidal, globular) [4].
- Stems are often ridged and glabrous (hairless)- rarely smooth and hairy and can be red, yellow, brown or green in colour [4,6,7].
- The main stem and the first branches become lignified with age (become "hard as wood") [3,4].
- When the plant is pruned, the buds at the base of the main stem break open and produce secondary stems [4].
- Alternating branches with petiolate (stalked) leaves from 1.5 to 10 cm long, very indented (bi-pinnate with linear serrated segments) [3,4].
- The leaves have a strong **characteristic aromatic odour** (fresh and bitter) due to the presence of glandular trichomes which secrete a volatile oil [2, 4,8].
- Alternate green leaves, generally glabrous (hairless) [7].
- Oval to triangular lamina<sup>1</sup>, deeply cut. Leaf edge generally dentate [7].
- Inflorescences<sup>2</sup> (flower clusters) of green-yellow panicles appear at the top of the main stem and branches [3,4,8].
- Tiny, yellow flowers arranged in capitula<sup>3</sup> (flower-heads) 2-3 mm in diameter in the inflorescences [8].
- Fruits are smooth, ovoid, light-grey **achenes**<sup>4</sup>, 0.5 cm long [3].
- Each fruit contains a single, **very small**, brown, oblong **seed** (less than 1 mm, i.e. 10.000 to 14.000 per gram) [3,4].
- Pollination is mainly by wind, less frequently by insects [4,8].
- Fertilisation mail allogamy (cross fertilization between two distinct plants) [4].
- Plant fertility (number of achenes per inflorescence) is highly variable [4].



Figure 2 : Artemisia annua leaf



<sup>&</sup>lt;sup>1</sup> Lamina : leaf blade

<sup>&</sup>lt;sup>2</sup> Inflorescence : cluster of flowers arranged on a stem

<sup>&</sup>lt;sup>3</sup> Capitulum, pl. Capitula : cluster of small flowers

<sup>&</sup>lt;sup>4</sup> Achene : Dry one-seeded fruit that does not open to release the seed

#### 4. Ecological requirements

• Day length and sun

Artemisia annua is a short day plant which starts to flower (and hence stops its growth) when the day length falls below a critical threshold value: between 11.5 - 13.5 hours, depending on the variety and growing conditions [4].

Hydric stress (excess or shortage of water), high temperatures, the physical impact of water on the plant and the wind can also induce a start of flowering [4].

Upon return of more favorable conditions, if daylight duration is not too short, the plant can stop blossoming and resume growth [4].

It is a heliophilous plant, which likes full sun when sufficiently supplied with water [4].

• Temperature

Germination of the seeds: from 7°C [1].

Optimal average growth temperature: 20-25°C [1].

Growth slows during the cold dry season [4].

The total temperature/days above the 10°C threshold must lie between 3500 and 5000°C to ensure good plant growth [1].

• Water

Artemisia annua requires significant amounts of water during the initial growth phase (young plants) but resists better to hydric stress thereafter. It requires a minimal rainfall of 600 to 650 mm/year to ensure growth. [9]

• Soil

Artemisia annua will grow best in not too heavy soils (i.e. sandy) with a pH between 5.5 et 7.5. It can grow in soil with pH below 5.5 but as a result will produce less biomass. [3]

It requires well drained soil as it doesn't not like waterlogging. [1].





Important: it is always possible to circumvent any adverse local conditions by selecting better suited varieties

#### 5. Phenology

#### 6 stades of development:

- 1. Seedling / rosette
- 2. Elongation and stem branching / pre-flowering
- 3. Formation of flower buds
- 4. Flowering
- 5. Fruiting
- 6. Senescence

There are overlaps of stages 3, 4, 5 and 6 depending on the parts of the plant [4].

**Cycle duration** varies according to plant variety and growing conditions. For this reason, it is important to identify optimum varieties and growing periods for your site. (See First Trial sheet: Which cultivar and which period?)

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## Artemisia afra



#### 1. Taxonomy

Artemisia afra Jacq. ex Willd is a species of the Asteraceae family. It has many common names, including "African wormwood", "wild wormwood" in English and "armoise africaine" in French.

Among these many local names are:

Wilde als, als, alsem (Afrikaans); Fivi (Kisambaa), Lunyaga (Kisafwa), umhlonyane (Swati, Xhosa, Zulu), um hlonyane (Xhosa), umhlorryane (Xhosa), msuzwane (Xhosa), mhlonyane (Zulu), iliongana (Tsawana), lengana (Pedi, Tswana, Sotho, Setswana), lusanje (Kinyakyusa), zengana (Southern Sotho); nthilili (Nyaneka), eliminiomba in Angola, aguppiyaa/agufa (Konta), yesiet kest (Amharic), ttcikkugne, ariti (Amaringa), chukun, jukun (Galinya-harar), kodo (Galinya), kapani (Galinya-bale), chugughee (Gedeoffa), godoguracha (Oromic) in Ethiopia; olchanipus, sisimwet (Sabaot) in Kenya; enjani pus, fivi/fifi (Swahili, Sambaa), injanev yoso, inyaga, linyaga, olunjanyioiboru, sumangara, fifi (Shambaa), ushemeli (Sukuma) in Tanzania.

[1-5]

#### 2. Origin and distribution

Artemisia afra Jacq. ex Willd is one of the longest known and most widely used medicinal plants in southern Africa.



It grows naturally in the mountainous areas of East and Southern Africa between 1500 and 3000 m altitude in Ethiopia, Kenya, Tanzania, Uganda, DRC, Zambia, Zimbabwe, Angola, Namibia, Botswana, Eswatini (formerly Swaziland), Lesotho, Malawi, Mozambique and South Africa.

It is the only native species (naturally native to this region) of the genus Artemisia.

[1,3-7]

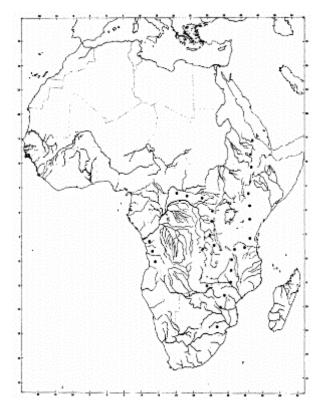


Figure 2 : Distribution Artemisia afra (FAO, 1986)

#### 3. Botanical description

The morphology of *Artemisia afra* varies enormously from one plant to another.

- Woody shrub, forming perennial **bushes** that vary in height from 0.6 to 2.4 m [8].
- Stems are pale green to grey, multiple, ridged and hairy [8,9].
- Thicker stems becoming woody at the base [10].
- Many smaller lateral branches grow from the main stems [10].
- Alternate, petiolate leaves, finely divided, similar to Artemisia annua 3-14 cm long and 1.5-6 cm wide.
- Oval lamina<sup>1</sup> with regular cut aspect. Even or dentate edge, slightly folded [8]
- The leaves are greyish -green and glabrous on the upper side, occasionally hairy [8].
- However, leaves are covered with small white hairs giving a felted aspect and **lighter green** in colour on the **lower side**.

These hairs also present on the stems give *Artemisia afra* **a characteristic "silver-grey" colour** which differentiates it from *Artemisia annua* [8-11].

- Caniculate leaf (the central ridge is slightly depressed on the upper side, and prominent on the lower side) [8].
- Easily identifiable aromatic odour [9].
- Pruning will give multiple branch growth.
- Inflorescences<sup>2</sup> in yellow-green panicles that appear on some branches of the plant.
- Tiny yellow butter-coloured flowers arranged in globular capitulums around 3 mm diameter [9,11].
- Fruits 1 mm long.
- Each ovary produces a very small achene<sup>3</sup>
- So far, obtaining viable seeds seems difficult.





Figure 3 : Artemisia afra leaf (left - upper side, right - lower side)



Figure 4 : Artemisia afra leaf (caniculate, hairy lower side)

<sup>&</sup>lt;sup>1</sup> Lamina : leaf blade

<sup>&</sup>lt;sup>2</sup> Inflorescence : cluster of flowers arranged on a stem

<sup>&</sup>lt;sup>3</sup> Achene : Dry one-seeded fruit that does not open to release the seed

#### 4. Ecological requirements



• Sun

Like Artemisia annua, Artemisia afra likes the sun. Wind appears to impact growth.

• Temperature

Average optimum growth temperature: 20-33°C [3].

Growth is slower in the cold season. Artemisia afra can withstand quite low temperatures in winter but dies under -7°C. [3,12]

• Water

Artemisia afra is more drought resistant than Artemisia annua once established. Water requirements thereafter are less frequent. [3]

It needs a rainfall of more than 650 mm/year to grow in abundance. In Tanzania, it occurs naturally in areas where rainfall varies from 900-2400 mm/year. [1]

• Soil

Artemisia afra is common on arid soil. In general, it is a hardy plant which grows well on any type of well-drained soil.

Growth is compromised if the pH is not between 5 and 7.5. [3]

It is found in upland wilderness in coastal areas or in steep areas, on wet slopes, along streams and on the edge of forests [9,11].

Important: it is always possible to circumvent any adverse local conditions by selecting better suited varieties

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#### 5. Phenology

#### 6 stades of development:

- 1. Seedling / rosette
- 2. Elongation and stem branching / pre-flowering
- 3. Formation of flower buds
- 4. Flowering
- 5. Fruiting
- 6. Senescence

There are overlaps of stages 3, 4, 5 and 6 depending on the parts of the plant.

Only certain branches will go through stages 3, 4, 5 and 6.

Due to its perennial nature, development is much slower than for Artemisia annua.

[12]

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## File 1 - Prerequisites for cultivation

Growing Artemisia is not simple. That is why certain conditions are required to start growing successfully.

#### 1. Cultivation site

Choose a flat sunny plot, preferably with loose soil. As far as possible, avoid sites on a slope, heavy clay or sandy soil, sites subject to flooding or with poor drainage and next to the sea.

WARNING! Do not cultivate in areas contaminated by hazardous substances (heavy metals, agrochemicals and other industrial waste). Avoid any risk of soil, air or water pollution. Evaluate the impact of past land use on the chosen cultivation site (previous plantations and possible applications of phytosanitary products in particular) [1].

#### 2. Access to water

WARNING! Growing *Artemisia annua* requires a significant amount of water since each plant must be watered generously morning and evening during the dry season. This amount of water must be reduced according to the rains during the rainy season.

The installation of a well or borehole and/or irrigation system is often necessary! It is however possible to cultivate *Artemisia annua* in the rainy season to make significant savings on water but this can give poorer results than in the dry season. The optimal growing period is to be defined according to each context.

Once well established, Artemisia afra is more resistant to high temperatures and drought. Its water needs are especially important during the first 3 months in the field.

Irrigation water must meet local, regional and/or national quality standards [1].



Irrigation water should not be contaminated by domestic animal or human materials [1]. (See AGRISUD Guide [7] p 85 to 87 - Protection of water against pollution).

WARNING: **Excess water** is also to be **avoided**! This can induce leaching of nutrients or reduction in root depth of the plant [2]. Moreover, **Artemisia annua is sensitive to waterlogging.** For this reason, drainage channels must be provided when growing Artemisia annua during the rainy season [3].

<u>For reference</u>: during the dry season at the House of Artemisia in Tivaouane, Senegal (hot and dry climate, Bsh, average 443 mm/year), plants are watered by sprinklers for 25 to 30 minutes morning and evening, every day for approximately one and a half months following transplantation and 15 minutes morning and evening thereafter. This represents 3.52 and 1.92 mm of water per day respectively.

The **choice of irrigation system** for each site must be determined according to its soil characteristics, topography, water quality and cost but also and above all according to the cultivation area and equipment available to reduce costs [4]. On small farms, using watering cans is often the most cost-effective system.

WARNING! It's important to reduce the physical impact of water on fragile seedlings to avoid destroying them [5].

At the Maison de l'Artemisia in Tivaouane, Senegal, the installation of a sprinkler irrigation system alone increased harvests by 50% compared to the drip irrigation used the previous year. It causes higher transplant mortality at the very beginning of the crop, but allows better decomposition of compost and mulch cover, leading to better growth of *Artemisia annua* [6].

To reduce the loss of young plants due to sprinkler irrigation, micro-irrigation is effective when plants are less than 1.2 m high [4].

In general, sprinkler irrigation seems to be more advantageous on sandy soil because roots develop on the surface to capture water that seeps into the soil very quickly; however localised drip irrigation is more appropriate on clay soil because roots are less extensive and go deeper to capture water retained in the soil.

(For more information, see AGRISUD Guide [7] p 75 to 84 - Water management).

#### 3. Equipment

- Fences if necessary;
- Watering system: watering cans, crop sprayers;
- Minimum 250 kg compost for a 200m2 trial (10 kg for the nursery and 240 kg for plants, ie 600 g/plant minimum);
- Mulch to cover the cultivated area;
- Basic gardening equipment: hoe, spade, shovel, machete, wheelbarrow, buckets, weeder, shears, sieve, ...);
- Nursery: wooden frames or honeycombed trays or cut cans, mosquito net or wire netting; protection against direct sunlight and rain;
- Small plastic bags or salvaged pots for possible pricking out before transplanting;
- Drying: tarpaulin (no holes), clean mats, drying table or other drying equipment;
- Shredding: machetes or hammer mill (16 or 24 hammers);
- Transportation (if the processing facility is off-site): clean bags;
- Seed collection: basins
- Storage of shredded material: clean, dry, hermetically sealed bags or boxes;
- Packaging: plastic-free kraft bags, dimensions 20\*8\*4 cm, with official Maison de l'Artemisia labels and stickers, single use latex gloves, clean gowns, masks, letter-weighing scale for measuring 40 g bags;
- Shred storage: bags or boxes tightly sealed
- Office supplies: notebooks or binders, pens, ...

#### 4. Crop manager

Growing Artemisia annua is not easy. Daily onsite presence and a minimum of agronomical knowledge are required in order to succeed in production of this plant. In addition, pesticide-free growing requires particular care to prevent and counter pests and diseases. Finally, crop rotation is essential, as is the control of other crops. (For more information, see AGRISUD Guide [7] p 133 to 137 - Crop Succession)

A crop manager must be appointed to meet the quality requirements for traceability of production batches.

We strongly encourage **agro-ecological practices** including **crop diversification**. (See AGRISUD Guide [7], in particular p 119 to 122 for the implementation of wind-breaking hedges and associations).



(See example of a typical House of Artemisia subtropical medicinal garden).

Advantages of crop associations: (For more information, see AGRISUD Guide [7] p 139 to 141 - Crop associations).

- Keep the soil alive and fertile ;
- Optimise use of growing space ;
- Reduce weeding, watering and improve soil by covering with mulch or creeping plants;
- NB: Artemisia growth is directly affected by weeds and soil fertility.
- Limit the use of inputs (water, fertiliser, phytosanitary products) ;
- Protect crops through the resilience effect of biodiversity;
- Improve production quality and quantity;
- Secure farmer income by diversifying production.

When choosing crop associations with Artemisia, consider complementarity:

- Layer crops (choose different strata of foliage allowing Artemisia to grow in the light);
- Choose plants that do not invade its poorly developed root system;
- NB: Artemisia has a pivotal root system that can draw water from deep down if necessary but does not descend if there is water on the surface as its secondary roots will develop in the upper soil horizon;
- Associate leguminous plants Artemisia is very demanding of nitrogen (eg: groundnuts, non-climbing beans, peas, soybeans, cowpeas, ... but avoid wheat, corn and cassava which also require a lot of nitrogen);
- Cultivate with other plants with similar water requirements [7].

Several conclusive trials have been reported with melons, peanuts, cabbages, non-climbing beans (planted after Artemisia) and lettuce (low competition for about 20 days and then harvested to let Artemisia grow, 20 cm spacing necessary).

Leave on site cut leaves of leguminous plants cultivated in combination to produce an excellent nitrogen-rich mulch!

Consider lemongrass and other plants produced to provide mulch.

WARNING! do not cultivate next to plants requiring chemical treatments!





It is vital to use only **non-toxic cultivation techniques because species of the genus Artemisia are powerful bio-accumulators**! [8] ie, they absorb and accumulate heavy metals (Cr, Ni, Co, Fe, Mn, Cu, Zn), chemical elements and radioactive waste.

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## File 2 - Compost Production

#### **Definition:**

Composting is an aerobic (requiring oxygen) biological process for the conversion and recycling of organic materials (biomass by-products, organic biowaste) into a stabilised, hygienic, soil-like product rich in humic compounds, which is called compost [1].

#### Advantages:

In addition to providing the necessary elements for plant growth, the addition of compost improves soil structure. It increases the humus rate and stimulates soil microbial life.

It deodorises animal manure and destroys certain microbial pathogens and weed seeds by a combination of temperature rise and biochemical degradation factors. It's a very important asset for organic farming.

A study in Senegal showed that adding compost to *Artemisia annua* plants reduced pest attacks (mainly soil fungi such as Rhizoctonia and termites) by half compared to the use of chemical fertilisers. It was also shown that the biomass yield is as good with compost (in particular compost made with nitrogen-rich poultry manure) as with the optimal chemical fertiliser in the study area [2].

Soil should be treated with well-fermented organic compost either before planting or immediately after the first harvest [3].

#### 1. Composting site

- Choose an easily accessible location, if possible sheltered from the sun, wind and water.
- It may be useful to erect a shelter to protect the compost from excess water and/or sunlight.

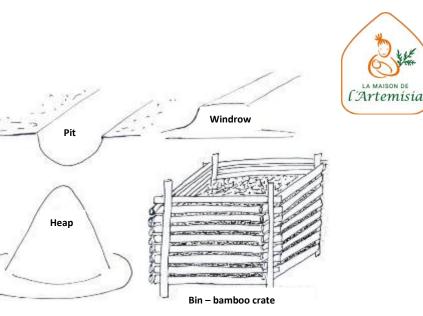


Figure 1: different types of composting (Agathe Cornet-Vernet)

# In wet areas, compost can be heaped in a pile 1m high x 1.5m diameter, in an air-permeable wooden crate (1m<sup>3</sup>), or in a windrow 1m high x 1m wide (length as needed) [4].

- In wet areas, dig water drainage channels around the compost heap.
- In arid areas, it is advisable to compost in pits to conserve moisture.
- Planting quickset hedging around the composter helps to maintain moisture and provides plant materials.
- Compost must always be in contact with the soil, which is the natural reservoir of organisms that allow materials to decompose.
- 4 spaces are generally necessary to turn the compost 3 times during maintenance.

#### 2. Preparation

- Make successive layers- see figure 2 : (indicative measurements) [4]
  - Dry organic materials (10 cm approx depending on size and density of material) : straw (recommended for mulching), grass, husks, dry leaves (except eucalyptus), peanut or palm kernels (in a thin layer because it takes longer to break down), cocoa waste, coconut waste and other dry plants. Cut/chop as necessary to speed up rotting process.
  - Fresh organic materials (5 cm) : vegetable matter, haulms, palms, tree leaves, peelings, ... either not too big or chopped. Do not use branches larger than 1 cm in diameter as they take too long decompose!
     NB: Consider also using plants rich in nitrogen (N) such as titonia or leguminous plants.
  - Animal manure : accelerate the rotting process with chicken droppings (2 cm), cattle, pig, donkey or horse dung, liquid manure, ... (2 to 5 cm).
     Increase the depth of fresh materials and animal manure if these are mixed with litter.
     NB: Chicken droppings are 3 times richer in nitrogen (N) than other types of manure.
  - Optional: thin layer of bone powder rich in phosphorus (P).
  - Optional: thin layer of ashes rich in potash (K).

For correct decomposition, a good C/N ratio (carbon/nitrogen) of materials used is important.

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Fresh organic materials Dry organic materials

Animal manure

Figure 2: compost layers (Agathe Cornet-Vernet)



- It is also possible to alternately layer dry organic materials (DM) with fresh organic materials (FM) or animal manure (AM): (DM-FM-DM-AM-DM-FM-DM-AM-DM...)
- Water well between each layer to start the decomposition process!
- Repeat successive layers to reach minimum height 1 m.
- Protect the surface from sun and wind and keep the compost moist by covering with straw, palm leaves, soil (in dry areas) or other local materials.

This is a standardised global method, but there are many ways to compost. For more information on the use of leguminous plants, manure and the different types of composting, see the AGRISUD Guide [4] p 89 to 114.

In Burundi, underground composting with E.M. technology (Effective Micro-organism by Prof. Jap. Teruo Higa) gives very good results. The compost is enriched with bacteria, molasses, sugar, water, but without the vinegar or alcohol (as provided for in the initial formula).

In Cameroon, Biochar is used successfully. For more information on this recognised soil improvement technique to improve water and nutrient retention and improve soil life, see the AGRISUD Guide [4] p 115 to 116.

In Burkina Faso, liquid fertiliser has been used successfully. For more information on this technique, which provides rich nutrients to crops at no cost. See the AGRISUD Guide [4] p 177 to 188.

**IMPORTANT:** To ensure the biological quality of the Artemisia crop, care must be taken to **use only ORGANIC materials**, free of any trace of chemicals. Animal manure must also come from an organic farm, which does not use antibiotics or feed with chemical additives because these would then transfer into the compost and ultimately into the Artemisia plants and consumed in herbal tea.

NB: *Artemisia annua* and *afra* appear to be very effective in treating coccidiosis in hens and parasitaemia in sheep (strongyles, strongyloids, coccidia and cestodes). Dried and ground Artemisia leaves and stems can be incorporated into feed in place of conventional medication. Studies report a dosage of 150mg of *Artemisia afra*/kg per day for 5 days for hens and 30g of *Artemisia afra* for sheep weighing 39kg on average for at least 8 days [5-7].

#### 3. Maintenance



2 weeks after assembling the layers, check that the rotting process is well underway by inserting a stick for 5-10 minutes into the middle of the compost. The stick must be warm (60 -70°C). [8]

If it is not the case:

- Wet any dry areas of the compost;
- Add fresh materials or animal manure as necessary (to redress the carbon/nitrogen ratio) [8].
- Water once a week in dry areas so the compost remains moist OR in case of drying out in wet areas.
- Monitor moisture regularly by checking a handful of compost from the middle of the heap and squeeze hard:
  - o If liquid trickles from the compost when squeezed it is too moist, so water less frequently;
  - o If no liquid trickles from the compost when squeezed and it crumbles when opening the fist, it is too dry and requires increased watering;
  - If no liquid trickles when squeezed, or only a few drops, and the compost remains compact when opening the fist, then moisture levels are correct.
- Monitor compost temperature regularly with stick method.
  - Turn the compost to another space (or pit) when it drops in temperature (stick method), generally every 2 4 weeks. This will maintain good aeration. After turning, the compost must be as homogenous as possible, with layers mixed and the edges turned into the middle.
- Water at each step to relaunch the rotting process with correct moisture levels (squeezed handful test).
- If the compost smells bad or if there are midges, poor aeration, excess water or excess of nitrogen rich materials (low C/N ratio) have caused fermentation and anaerobic moist areas.

To correct this, turn the compost and add carbon rich materials.

#### 4. Completion

- The compost is ready when it is homogeneous (the original materials can no longer be distinguished) and does not heat up, 2 to 4 months in dry areas, 3 weeks in wet areas.
  - It should be light, moist and aerated, dark brown in color and smell like forest soil.
- Sieve coarsely before use to remove any un-decomposed material (wood, stems, ...) which can be recycled in the initial preparation of the next compost.

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## File 3 - Growing trial: choice of cultivar and growing period

There are several cultivars of *Artemisia annua*. It is difficult to know in advance which is best suited for a specific biotope and the optimal growing period. The growing trial will therefore test seeds from several sources to determine which works best for a given context. The optimal growing period is when days are long, water is available and harvesting outside of the rainy season is possible.

#### **Objectives** :

- What and when to plant? Identify the most suitable seeds and the best growing period.
- Choose the best seed suppliers! Collect seeds from the most suitable plants.

#### 1. Schedule for growing trial

- Ideally, start new seed beds so you can transplant each month in the field 1 row of 20 new plants for each cultivar.
- If the frequency (sowing and transplanting each month) is too demanding, choose four dates based on the seasons.
- If the number of plants required is difficult to manage, transplant 1 row of 15 new plants for each cultivar on each date.

#### 2. Site selection

- Make sure that the site is uniform (same soil type, sun exposure, water supply, ...) and as flat as possible.
- If the ground is not flat and there is risk of waterlogging, plant lines in the direction of the slope to facilitate drainage.

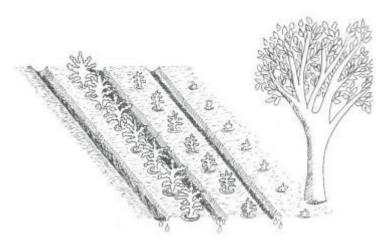


Figure 1 : Sloping trial site (Agathe Cornet-Vernet)



- If the site is not uniform, plant rows in such a way as to expose them all equally to the source of variability

Example 1 : The test plot is bordered by a row of trees to the east.

If rows are planted in a north-south line, the row closest to the trees will be impacted by shade and wind protection and the soil will be enriched in organic matter from fallen leaves...

This row of trees is a source of variability. It will influence plant growth in the same way as the choice of period or cultivar, resulting in biased test results.

Planting rows in an east-west line allows this variability factor to be "distributed" over each test row and thus not distort results.

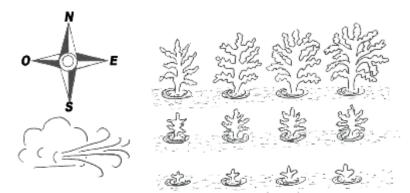


Figure 3 : Trial site with strong crosswind (Agathe Cornet-Vernet)



Example 2 : If the trial site is subject to a strong crosswind from the west, plants on the west of the site will be impacted and will have more difficulty growing.

Rows should therefore be planted in east-west lines. If rows are planted in north-south lines, one could incorrectly assume that the cultivar or period trialled are to be blamed for poor growth when it is just a question of the impact of the wind.

- If several plots or fields are available, carry out the test in different locations (1 row of 20 plants per cultivar and per date).

This is the principle of repetition: two plots of equal area, the same number of viable plants, the same cultivar and the same transplantation date that are geographically separated will give much more representative results than one plot. This will enable possible differences between plots to be taken into account and spread the risk of pests or other unexpected issues.

#### 3. Cultivation operations



#### See Cultivation and Processing Manual.

Important : clearly delimit the plant beds for each cultivar! Use wooden boards to separate beds and write on the cultivar name of each zone.

- Transplant to the same density and use the same quantity and quality of water and compost for each trial!
   Density, water and compost are sometimes more important factors for growth than cultivar or growing season.
- Weed if possible with the same regularity after transplantating; as soon as necessary in the first weeks and then approximately every month.
- DO NOT USE ANY PRODUCTS, organic or not, as the objective here is to see which cultivar is best suited to local conditions.
- Harvest once only (one final cut). The objective of the growing trial is to identify which cultivar is best adapted to the growing site for each period of the year and see if the cultivar flowers prematurely, rather than to produce as much herbal tea as possible by regular cutting.

#### 4. Observations

- Note cultivar, sowing and transplanting dates for each row in a notebook.
- Note information on the trial site (sector + landmarks + possible variability factors for each row) and the number of plants in each row.
- Carefully observe plant growth and check each week for pests, disease or other abnormalities.
- Determine and record **pest attacks** with **description** and evaluation of the **infestation rate** (% of affected plants on the plot), **impact** (evaluation of lost production per affected plant), and **mortality rate** (% of dead plants).
- <u>When half the plants have formed flower buds</u>, measure all the plants in the row EXCEPT those that have been attacked by pests or disease (ie with estimated 50% or more loss of production or impacted over 50%).
- Record height of the smallest plant (minimum height), the largest plant (maximum height) and the average height (sum of heights of all the plants ÷ number of plants in the row) taking into account only the plants that have not been seriously attacked by pests or disease.
- Record date of cutting (to be done as soon as the flower buds appear on half of the plants in the row, after taking measurements).

IMPORTANT: Do not cut the best plants but leave them to flower for seed collection. (See: Seed Production)

- If possible, dry each row separately in order to weigh and note the **dry material production per unit (row and zone**) and calculate the **average dry material produced per plant** (taking only into account plants that have not been badly attacked by pests and disease).



#### Trial log: (example)

Cultivar	Sowing date	Transplan- tation date (T)	Zone(s) – n°plants	Variability factors	Density	Compost	Pests and disease attacks	Observations min, max and average height just before harvest	Harvest (per zone) - date + grams of <u>dried plant</u> <u>material</u> + average yield dried
Senegal	08/10/2017	24/11/2017	A - Row 1 (20 plants) B - Row 5 (20 plants) C - Row 3 (20 plants)	A - Windy B- Close to village, sloping ground C – Partly under trees (shade, leaf humus, nutrients and water pumping	0,5 x 1 m	3 handfuls of poultry manure compost/ plant at T	A - 50 % of plants with ants : minor impact B - 30 % of plants with termites on roots: 10 % dead, 5 % weakened, 5 % ok C - 20 % fungi appeared on 15/04/18 on a few leaves (yellow/orange/brow n) causing leaves to dry. 40 % affected on 30/04 : 20 % with black stems $\rightarrow$ removed from the field 20% affected leaves (about 1/5 <sup>th</sup> of the plant) $\rightarrow$ leaves removed	A - Early flowering! Wind?Min H:83 cmMax H:163 cmAvg H:124 cmB - Weak growing at first poorer soil?Add more compost?Min H:140 cmMax H:191 cmAvg H:161 cmC - 5% malformation, very small ecotype, « round shaped », not very productive.Min H:77 cmMax H:146 cmAvg H:126 cm	<ul> <li>plant material per plant</li> <li>A - 05/03/2018</li> <li>⇒ 5 343 g/row <ul> <li>(÷ 20 plants per row)</li> <li>⇒ Average 267 g/plant</li> </ul> </li> <li>B - 25/05/2018 <ul> <li>⇒ 10 012 g/row</li> <li>(÷ 17 plants per row)</li> <li>(2 plants died of termites And one kept for seed!)</li> </ul> </li> <li>⇒ Average 589 g/plant</li> <li>C - 13/05/2018 <ul> <li>⇔ 6 569 g/row</li> <li>(÷ 16 plants per row)</li> <li>(4 plants died of fungi)</li> </ul> </li> <li>Average 411 g/plant</li> </ul>

#### 5. Seed selection (cultivar and period)



- Plants that are less vulnerable to pests and diseases.

Selection of a resistant cultivar = most effective means of control and always the preferred option! Even if yield is slightly lower than with another non-resistant plant, it will avoid considerable costs and production losses when pests or disease appears again.

- Maximum production (average dry weight per plant).
- High germination rate in seedbed (with fast germination.

#### Example of optimal growing season in Senegal (for information)

At the House of Artemisia in Tivaouane, years of testing have made it possible to establish the following growing schedule:

Sowing is done at the end of the rainy season (October) so as to transplant the plants to the field as early as possible in the dry season (November).

Plants are irrigated and cut once the largest plants reach just over 1 m high. The final harvest takes place just before the rainy season (June) to facilitate drying. Over the 6 growing months, usually only one intermediate cut is made.

It is also possible to cultivate in the rainy season (transplanting in July, manpower permitting). In this case, only one final cut is made 3 months later, at the end of the rainy season.

## File 4 - Sowing



IMPORTANT! This process is extremely delicate. Plants are very fragile for a month after germination.

#### 1. Preparing the seed bed

- Cut a plastic canister in half and make small perforations in the bottom **OR** use a seed tray with drainage holes **OR** use wooden planks (approx 30cm deep) to make a free-standing seed bed in open ground.
- Shelter from rain and direct sun making sure seeds have plenty of light (from the sides).
- Prepare the seed bed with the following mix :
  - 1/3 well-decomposed compost
  - 2/3 local soil (OR 1/3 local soil + 1/3 sand if local soil is too heavy)
- Sieve to obtain a light and fine mix. Remove any large or coarse particles that could hinder seed germination.
- Sterilise the soil to destroy weed seeds, pathogens (eg. damping off fungal disease) and larvae that could adversely affect seed development.

Method 1 - boiling water sterilisation : use a watering can and water with boiling water

**Method 2** - solar sterilisation : dampen soil, cover with black tarpaulin and leave in direct sun for 5 days (maximum soil depth 15 cm).

**Method 3** - heat treatment : heat the substrate over a fire for 15 minutes, turning regularly, then leave 24 hours to rest before use.

#### No pesticides will be needed with properly sterilised soil.



Figure 1 : half canister seed bed (Agathe Cornet-Vernet)

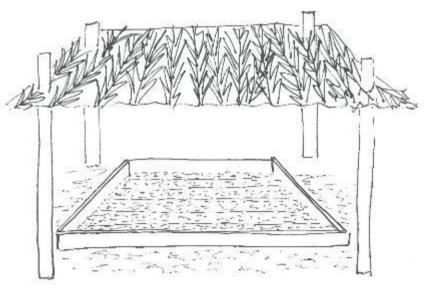


Figure 2 : seed bed (Agathe Cornet-Vernet)

However, if treatment is required to combat insect pests, use a neem based solution (1%).



#### 2. Sowing

Our experience indicates that a 1 m<sup>2</sup> seed bed using seed produced by the Maison de l'Artemisia network will yield approximately 200 plants.

- Water the seeding area.
- Shake the packet to loosen the seeds and measure out 1 teaspoon of seed.
- A study carried out in Nigeria in 2014 recommends soaking seeds in hot water (60°C) for 2-3 minutes to reduce the germination period and ensure stronger seedlings. The best germination rate was observed when seeds were soaked in sulphuric acid (10%). However, soaking in hot water is both easier and cheaper. [1]
- Mix 1 teaspoon of seed with 10 teaspoons of sieved sand to dilute the seeds if they are clean. If the seeds have not been winnowed and are mixed with bits of flowers, then reduce the amount of sand accordingly.

Adapt the amount of sand if necessary for homogenous sprouting at optimal density (based on germination rate of seeds).

- Gently sprinkle the seed/sand blend in lines, in wide movements, over the whole seed bed, paying attention to the wind direction.
- Use a fine spray or water mister to fix the seeds.

You can use a clean sprayer that has not previously been used to apply chemicals or a watering can with a spray head. Alternatively, moisten a broom and shake to create a rain of small droplets. Do not use a watering can without a spray head.

IMPORTANT! Seeds must remain on the surface to germinate. Hold can or mister sufficiently high to avoid disturbing or moving the seeds.

- Water morning and evening with a mister or very fine spray until transplanting OR use a capillary watering system by placing the perforated half canister in a non-perforated half canister halffilled with water (figure 5).
- IMPORTANT : moisten the soil only. Do not swamp the soil or the seedlings will rot!

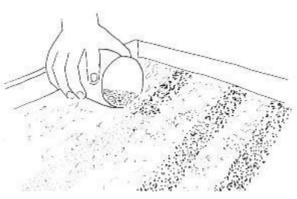
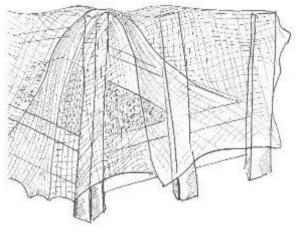


Figure 3 : sowing method (Agathe Cornet-Vernet)





- Cover with wire netting or untreated mosquito net after watering. Raise netting on sticks placed around the seedbeds to protect from pests.

→ Appearance of "first two round leaves" followed by other toothed leaves (2 to 5 days if germination is good - up to 2 months).

Remove all weeds by hand or with a hoe!

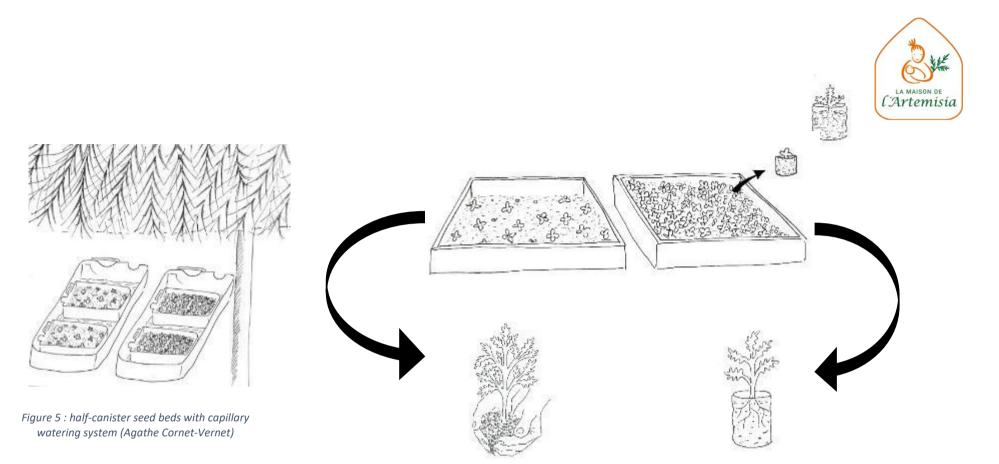
Figure 4 : covered seed bed (Agathe Cornet-Vernet)

Seed bed growth :

- With proper spacing between seedlings ( > 1 cm), leave seedlings to grow to 10 cm high.
- If spacing is too small, separate seedlings once they reach 3 to 4 cm high and grow in individual pots.

Small plants can be transplanted once they reach a hand's length ( (10 to 15 cm high).

Plants are hardy once about 10 leaves have formed and they can survive in a small space before being planted out.



*Figure 6 : seed bed in open ground and transplanting depending on density of seedlings (Agathe Cornet-Vernet)* 

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## File 5 - Cultivation Artemisia annua

The principles of good agricultural management, including appropriate crop rotation according to environmental requirements, must be respected, and ploughing must be adapted to plant development and other crop needs. Where appropriate, conservation agriculture (agro-ecology) should be used, particularly with regard to the accumulation of organic matter (compost, mulching) and conservation of soil moisture (mulching, sustainable irrigation). [1]

Farmers should adopt practices that contribute to soil conservation and reduce erosion, such as creating buffer zones along watercourses, planting cover crops and adding green plant matter (to be incorporated into the soil when ploughing) [1].

These agro-ecological techniques are described in the AGRISUD Guide [3].

#### 1. Preparation of the plot

IMPORTANT: Prepare the plot for transplanting at the same time as the seedbed. These operations are labor intensive and can be time consuming. They should be done about 2 months before planting.

- If possible, fence off the growing area to avoid damage from wandering animals. Cattle should not be allowed to enter the growing area [1].
- Clear, harrow and surface plough only if necessary. Avoid slash-and-burn agriculture which destroys soil life!
- Rake to remove stones and weeds.
- Apply organic fertilisers if the soil is poor (see AGRISUD Guide [3] p 97 to 103 Organic manure).

WHO recommends that soil should contain appropriate amounts of nutrients, organic matter and other elements [1].Ideally, a soil test should be performed and the results recorded in the batch and cultivation record.Add manure to the soil, preferably 2 weeks prior to transplanting.This does not dispense you from adding compost and manure as part of regular plant care.

- Hoe to loosen the soil and form cultivation beds or ridges as best.
- Dig furrows (drains) to drain the soil if the crop is grown in the rainy season.

#### 2. Transplantation

- Make a hole approximately one hand deep (approx 20 cm, depending on the roots).
- Water the hole generously to loosen the soil and facilitate transplanting and recovery of young plants (in an intensive cultivation system with irrigation, irrigate the plot for 4 hours, about 15mm).
- Crumble the soil well and mix in a large handful of compost (approx 200 g).

**Use of mineral fertiliser (including urea and NPK) is not allowed in organic farming!** As nitrogen (N) is a determining factor in the growth of *Artemisia annua*, it is possible to add ground horns (from slaughterhouses) to compost.

- Water the seedbed to loosen the soil before removing the plants.
- Transplant the seedling, keeping a clod of earth around the roots!
- Apply 2 large handfuls of compost in a circle around each plant to promote lateral root growth. IMPORTANT: Do not cover the leaves to avoid the risk of burning!



Artemisia

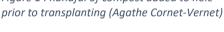




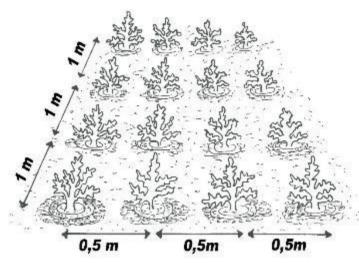
Figure 2 : transplanting an Artemisia seedling (Agathe Cornet-Vernet) The doses to be applied are the same as those traditionally used in market gardening. The staggered application of 1 kg of poultry manure compost per plant gives very good results. It is essential to adapt the type of manure taking into account the specific growing conditions of the region: soil type, climate, possible irrigation. Nitrogen appears to be the determining nutrient in the growth of *Artemisia annua*. [2]

IMPORTANT: Nutrient inputs differ according to the type of compost. Compost made with poultry droppings provides about 3 times more nitrogen than compost made with donkey manure, cattle manure, pig manure or green waste. Triple doses of non-poultry manure compost!

Manure must be carefully decomposed to meet health standards for acceptable limits of microbial contamination and to destroy the germination capacity of weeds. Human excrement should not be used as fertiliser because of the possible presence of infectious microorganisms and parasites. Any application of manure should be documented. [1]







*Figure 3 : Artemisia plot (Agathe Cornet-Vernet)* 

#### Optimal planting density depends on several factors:

Variety, soil type, period of the year, manure used, association or not with another crop, technical itinerary, ... [2]

Planting at a density of **2 plants/m<sup>2</sup> (100 x 50 cm)** in lines 1 m apart with 50 cm between plants to promote growth and limit the occurrence of fungal disease has proved best in Senegal under favourable conditions (fertile soil, no water stress, vigorous genotype and not too short day length) [2].

#### In less favorable conditions, density can be increased to 4 plants/ $m^2$ (50 x 50 cm).

We advise against monoculture over a large area. These measures should be adapted by alternating Artemisia with market gardening and subsistence crops.

#### 3. Mulching

Mulching the lines with fresh or dry organic matter (green plant waste) helps limit the need for water and weeding, reduces soil erosion, provides additional nutrients and prevents the soil from dirtying the lower branches of the *Artemisia annua* plants.

Use any type of straw, tops, grasses, cereal crop residues, cut or crushed corn plants, decomposed palm oil leaves ... Avoid wood chips from the sawmill as they are too acidic. Favour local reclaimed products! (See Guide AGRISUD [3] p 143 to 144 - Mulching).

For example, citronella can be pruned every 15 or 21 days to produce the necessary mulch.

A system of cultivation on plant cover or using cover plants can also be considered (see Guide AGRISUD [3] p 197 to 207).

#### 4. Plant care



Water each plant generously morning and evening every day using a watering can, sprinkler or drip.
 Irrigate early in the morning and late in the evening or at night to reduce evaporation and consequent water loss.

IMPORTANT: Do not flood the soil, but moisten it well. Artemisia annua is sensitive to waterlogging and water stress is fatal to young plants, causing early flowering and consequently stopping plant growth!

- Weed regularly after transplanting and then every month.

IMPORTANT: remove all weeds as Artemisia annua is very sensitive to competition!

- Monitor plants regularly and act quickly in case of disease (such as the appearance of mould if watering is too heavy) or pests (goats, rabbits, cattle, termites, grasshoppers, ...)!
- Replace dead plants several times during 2 months.
- If necessary, provide shelter from the sun or wind when young plants are first planted out.
- Do not worry about variations in the shape and height of the plants.

After 2 weeks in the field, when the plants reach a height of 30 to 50 cm, plants can be **coppiced or pruned** by cutting 5 to 10 cm off the top. Topping will produce more productive plants with more branches. This harvest can be used for its health benefits as well as the final harvest. [4]



Figure 4 : watering an Artemisia plant (Agathe Cornet-Vernet)

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# File 6 - Harvesting Artemisia annua

#### Important information:

One plant can produce up to 1.5 kg of fresh leaves and stems giving 375 g dry material per plant.

Artemisia annua grows stronger after cutting. A study in Benin has shown that **topping (coppicing) results in more productive plants with more branches.** A study in Senegal demonstrated that an **intermediate cut can harvest up to 2 times more biomass** and is more profitable than a single final cut with the right plant growth! [1,2]

Harvests from coppicing/pruning and intermediate cuts are processed and used for their health benefits as well as the final harvest. According to feedback from our network, and considering the fact that Artemisia herbal tea is a polytherapy, there appears to be no loss of efficacy with harvests made throughout the entire elongation stage of the plant. Since 2016, herbal tea harvested just 2 weeks after transplanting has been used successfully to treat malaria in Benin. The constituents of the plant change during other phenological stages.

IMPORTANT! Artemisia annua is rich in active ingredients and is therefore effective when harvested <u>before and during flower production</u>. Flower buds are very small, round and green. Flowers are very small, yellow and delicate. Once the flowers wilt and during fruiting, the active molecule content in the plant drops rapidly! The plant should not be used for medicinal purposes from this point !

IMPORTANT: Leave the finest and most productive plants to flower to collect their seeds! (See File - Seed Production). Identify plants to be reserved for seed collection before the final cut and do not harvest them for herbal tea production.

WHO strongly advises against harvesting in the rainy season because excess moisture promotes microbial fermentation and mould growth! [3]. The Maison de l'Artemisia recommends adjusting the growing calendar to harvest in the dry season. (See File Growing Trial : choice of cultivar and growing period).

The harvest should be carried out in the driest possible conditions (ideally in the middle of the day) and plant material should be dried immediately. [3]

All hygiene rules must be strictly complied with.

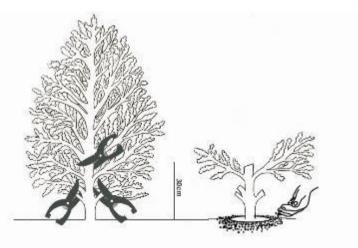


**1. Coppicing/pruning:** After 2 weeks in the field, when the plants reach a height of 30 to 50 cm, plants can be **coppiced or pruned** by cutting 5 to 10 cm off the top. This topping will produce more productive plants with more branches. This harvest can be used for its health benefits as well as the final harvest.

# 2. Intermediate cut: About 2 months after transplanting, when the <u>tallest plants</u> in the field reach 1 m in height.

NB: This height indication is for guidance only. Artemisia plants vary enormously in height within the same field. It is not necessary to wait until they all reach 1 m to cut. Some will be only 80 cm or 40 cm but the height of the largest plants justifies harvesting.

- In the dry season, if there is a lot of dust and soil on the plants, rinse them in the morning of the day before harvest by watering abundantly with clean water.
- In a dry climate, cut the plants to 30 cm high with clean and dry secateurs.
   IMPORTANT: DO not cut any lower or the Artemisia will die!
- Harvest any lower branches (which will turn yellow quickly). IMPORTANT: Rinse thoroughly with water if they are dirty!
- Do not harvest yellow leaves that dry out or leaves damaged by fungi, pests and other diseases.
- Spread 2 large handfuls of compost around the base of the plant to promote the recovery of development after cutting.



*Figure 1: Coppicing/pruning Artemisia annua in a dry climate (Agathe Cornet-Vernet)* 



#### IMPORTANT: This method doubles yield in the dry season but does not appear to work well in wet climates!

Cutting to 30 cm in wet climates encourages development of diseases. In these conditions it is therefore recommended to **lightly prune back** outer branches purely to encourage ramification to increase biomass production.

**Several intermediate cuts are possible** before the final harvest if the plants again reach a height justifying another harvesting (1 m high for tallest plants in the field) and if the climate allows for the crop to stay in the field for another 2 months.

- **3.** Final cut: approximately 5 to 6 months after transplanting, during flowering **OR** after the optimal drying period.
  - In the dry season, if there is a lot of dust and soil on the plants, rinse plants in the morning of the day before harvest by watering abundantly with clean water !
  - Cut the plants at the base with a clean and dry machete.
  - Remove roots from the field as they reduce yield of subsequent crops (due to their allelopathic effect).
     NB : Roots can be composted or used to produce a mother tincture to aid digestion. To make the tincture, dry-clean the roots with a wire brush or scrubbing brush. Roots can also be soaked in water and brushed to remove any soil. Wipe off excess moisture and leave to dry. Macerate for at least 3 weeks in alcohol of choice.
  - For seed plants, reduce watering by half after flowering to encourage the production of germinating seeds.

Care should be taken to ensure that no foreign matter, weeds or toxic plants are mixed with the harvested medicinal plant material. Harvested material that is damaged or decomposed should be retrieved and disposed of during and after harvesting to avoid microbial contamination and loss of product quality. [3]

Harvested plant material must not be piled up on the ground. Contact with soil should be avoided to minimise the microbial load of harvested medicinal plant materials. If necessary, large pieces of clean cloth can be laid on the ground to protect the crop. [3]

Harvested plant material must be collected in containers such as bags, baskets, wheelbarrows or trailers that are clean and dry. Residual moisture and possible contamination by soil or other materials must also be avoided. [3]

Equipment should be stored in a dry, pest-free place out of the reach of livestock and pets [3].



Mechanical damage or compaction of raw medicinal plant materials, such as from overfilling or stacking of bags, which may lead to spoilage or other loss of quality, should be avoided. [3]

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# LA MAISON DE L'Artemísía

# File 7 - Cultivation Artemisia afra

The principles of good agricultural management must be respected. Where appropriate, conservation agriculture (agro-ecology) should be used, particularly with regard to the accumulation of organic matter (compost, mulching) and conservation of soil moisture (mulching, sustainable irrigation). [1]

Farmers should adopt practices that contribute to soil conservation and reduce erosion, such as creating buffer zones along watercourses and planting cover crops and green manure (to be incorporated into the soil when ploughing) [1].

These agroecological techniques are described in the AGRISUD Guide [2].

# 1. Choice of where to plant

IMPORTANT: As Artemisia afra is a perennial bush, think carefully about where to plant as its location will be permanent! Once it has been in the ground for several months, it does not tolerate well being transplanted elsewhere.

Allow for at least 1 m between the Artemisia afra plants because they grow at least 1 m wide.

For example, *Artemisia afra* plants can be planted in lines with plants 1 m apart so that they touch each other, with a 2 m gap before the next line to allow for layering and passage for harvesting.

Adapting these measurements by alternating the Artemisia with trees, market gardening and subsistence crops is strongly recommended.

It is also quite possible to grow individual plants in pots or in the open ground.



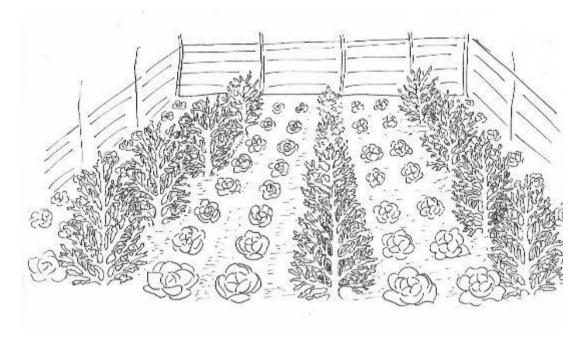


Figure 1: Artemisia plot with mixed crops (Agathe Cornet-Vernet)

#### 2. Preparation of the plot

IMPORTANT: Prepare the plot for transplanting at the same time as the seedbed. These operations are labor intensive and can be time consuming. They should be done about 2 months before planting.

- If possible, fence off the growing area to avoid damage from wandering animals.

Cattle should not be allowed to enter the growing area [1].

- Clear, harrow and surface plough only if necessary. Avoid slash-and-burn agriculture which destroys soil life!
- Rake to remove stones and weeds.
- Apply organic fertilisers if the soil is poor (see AGRISUD Guide [2] p 97 to 103 Organic manure).

WHO recommends that soil should contain appropriate amounts of nutrients, organic matter and other elements [1].

Ideally, a soil test should be performed and the results recorded in the batch and cultivation record. Add manure to the soil, preferably 2 weeks prior to transplanting. This does not dispense you from adding compost and manure as part of regular plant care.

- Hoe to loosen the soil and form cultivation beds or ridges as best.
- Dig furrows (drains) to drain the soil if the crop is grown in the rainy season.

# 3. Transplanting

- Make a hole approximately one hand deep (approx 20 cm, depending on the roots).
- Water the hole generously to loosen the soil and facilitate transplanting and recovery of young plants (in an intensive cultivation system with irrigation, irrigate the plot for 4 hours, about 15mm).

determining factor for growth it is possible to add ground horns (from slaughterhouses) to compost.

Water the seedbed or propagation site to loosen the soil before removing the plants.

Crumble the soil well and mix in a large handful of compost (approx 200 g).

Transplant the seedling, keeping a clod of earth around the roots! Make a small basin around the plant to keep water close to its roots.





Figure 2: handful of compost added to hole prior to transplanting (Agathe Cornet-Vernet)

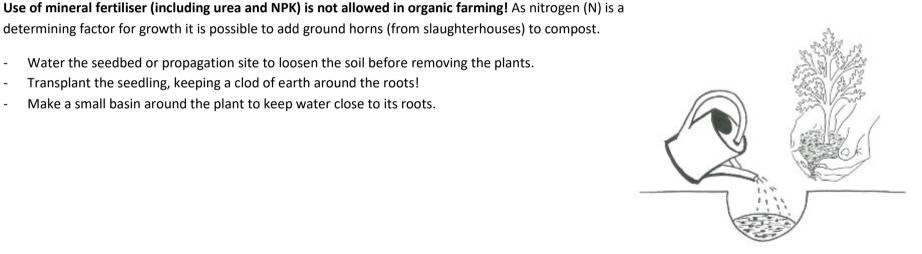


Figure 3: transplanting an Artemisia seedling (Agathe Cornet-Vernet)

### 4. Mulching



Mulching the lines with fresh or dry organic matter (green plant waste) helps limit the need for water and weeding, reduces soil erosion and provides additional nutrients.

Use any type of straw, tops, grasses, cereal crop residues, cut or crushed corn plants, decomposed palm oil leaves ... Avoid wood chips from the sawmill as they are too acidic. Favour local reclaimed products! (See Guide AGRISUD [2] p 143 to 144 - Mulching).

For example, citronella can be pruned every 15 or 21 days to produce the necessary mulch.

A system of cultivation on plant cover or using cover plants can also be considered (see Guide AGRISUD [2] p 197 to 207).

#### 5. Plant care

Artemisia afra is very fragile prior to planting out. The first few months are crucial for it to become strong and lignified.

- Water each plant generously morning and evening every day using a watering can, hose, sprinkler or drip for the first 3 months. Irrigate early in the morning and late in the evening or at night to reduce evaporation and consequent water loss.

3 months after transplanting the Artemisia afra, if it is properly established, watering can be limited to 2 or 3 times a week.

IMPORTANT: Do not flood the soil, but moisten it well. Reduce watering according to rainfall during the rainy season.

- Weed regularly after transplanting and then every month as required.
- Add compost after each weeding

For best results, add 1 handful of compost 1 month after transplanting, 2 handfuls 2 months after transplanting, 3 handfuls 3 months after transplanting and 4 handfuls 4 months after transplanting.

IMPORTANT: Do not cover the leaves to avoid the risk of burning! Spread the compost in a circle around each plant.

It is essential to adapt the type of manure taking into account the specific growing conditions of the region: soil type, climate, possible irrigation.



IMPORTANT: Nutrient inputs differ according to the type of compost. Compost made with poultry droppings provides about 3 times more nitrogen than compost made with donkey manure, cattle manure, pig manure or green waste. Triple doses of non-poultry manure compost!

Animal manure must be carefully decomposed to meet health standards for acceptable limits of microbial contamination and to destroy the germination capacity of weeds. Human excrement should not be used as fertiliser because of the possible presence of infectious microorganisms and parasites. Any application of manure should be documented. [1]

- Monitor plants regularly and act quickly in case of disease (such as the appearance of mould if watering is too heavy) or pests (goats, rabbits, cattle, termites, grasshoppers, ...)!
- If necessary, provide shelter from the sun or wind when young plants are first planted out.
- Do not worry about variations in the shape and height of the plants.

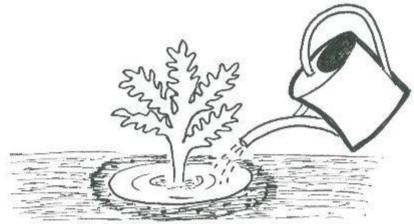


Figure 4: watering an Artemisia plant (Agathe Cornet-Vernet)

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# File 8 - Harvesting Artemisia afra



# 1. Harvesting conditions

It is very difficult to estimate Artemisia afra yield but one plant should provide curative and preventive treatments for several people!

One plant can produce 500g - 1.5kg dry leaf material per year with 3 to 4 cuts each year.

Artemisia afra, just like Artemisia annua, develops branches and grows better after cutting. Pruning and coppicing therefore provides branches to be dried for herbal tea and encourages ramification which leads to increased biomass production.

IMPORTANT: Do not use flowering branches for the production of herbal tea.

# IMPORTANT: Leave the finest and most productive plants to flower to collect their seeds! (See File - Seed Production).

WHO strongly advises against harvesting in the rainy season because excess moisture promotes microbial fermentation and mould growth! [1]

# Artemisia afra can be harvested throughout the year. Yields are higher in the rainy season.

The harvest should be carried out in the driest possible conditions (ideally in the middle of the day) and plant material should be dried immediately. [1]

All hygiene rules must be strictly observed!

2. Harvesting: once *Artemisia afra* is over 50 cm high, plants can be pruned very lightly for the first time. Plants may reach this height in the first month after transplanting, but sometimes it is necessary to wait over 3 months depending on growing conditions. The first harvest is small but will encourage ramification of the plant which will ensure a much higher production later on. Once established, this perennial plant will be more hardy and provide greater yields.



- In the dry season, if there is a lot of dust and soil on the plants, rinse plants in the morning of the day before harvest by watering abundantly with clean water!
- Cut off the year's growth with clean, dry secateurs.

Artemisia afra can withstand cutting if the tools are properly sharpened and do not damage the woody stems [2].

IMPORTANT: Do not cut lower than 50 cm or the Artemisia afra will die!

# Keep low hanging branches for layering! (See file Layering)

- Add 2 large handfuls of compost around the base of the plant to promote the recovery of development after each cutting.



Figure 1: pruning Artemisia plants (Agathe Cornet-Vernet)



# New growth can be pruned as soon as the plants reach a good size again and the climate is suitable for correct drying.

Care should be taken to ensure that no foreign matter, weeds or toxic plants are mixed with the harvested medicinal plant material. Harvested material that is damaged or decomposed should be retrieved and disposed of during and after harvesting to avoid microbial contamination and loss of product quality. [1]

Harvested plant material must not be piled up on the ground. Contact with soil should be avoided to minimise the microbial load of harvested medicinal plant materials. If necessary, large pieces of clean cloth can be laid on the ground to protect the crop. [1]

Harvested plant material must be collected in containers such as bags, baskets, wheelbarrows or trailers that are clean and dry. Residual moisture and possible contamination by soil or other materials must also be avoided. [1]

Equipment should be stored in a dry, pest-free place out of the reach of livestock and pets [1].

Mechanical damage or compaction of raw medicinal plant materials, such as from overfilling or stacking of bags, which may lead to spoilage or other loss of quality, should be avoided. [1]

# Bibliography:

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# File 9 - Pest control



The term "pests" refers to **animals, insects** and **diseases** that attack crops.

### 1. Methodology

- Observe plants in each plot at least once a week and every day if pests appear.
- Act as quickly as possible at the slightest anomaly.
- Determine the cause (termites, fungus, irrigation, wind etc.).
- **Evaluate the impact on total harvest** by calculating the percentage of affected plants whose biomass production is seriously reduced (= number of affected plants with seriously reduced biomass production / total number of plants on production site x 100%).
- Always prioritise **preventive measures** : seed suitability, biodiversity, crop associations, crop rotation, use of fully mature compost, mulching, adequate watering, non-infested equipment etc.
- In the event of an infestation with a disease that seems to be spreading, immediately remove parts of plants or entire affected plants from the field and burn. Do not compost!

#### IMPORTANT: Plant protection products are PROHIBITED in Organic farming! Plant growth regulators are prohibited too.

Agrochemicals used to promote the growth of medicinal plants or to protect them must be applied in minimal quantities and only if there is no other option. **Integrated pest management methods** for crop pests, i.e. favouring natural mechanisms and the use of pesticides, should only be used if economically justified and safe for human health and the environment! [1]

#### For more information on integrated pest management and different control methods, refer to the AGRISUD Guide [2] p 145 to 148.

- Only treat if the pest is uncontrollable, has significant impact, causes real losses and it is cheaper to apply a plant protection product.
- Choose a product approved for organic production, for consumer crops and in compliance with the regulatory requirements of the country of production and consumption of the end product [3].
- **Follow instructions on the packaging** or package leaflet of the plant protection product used, including the pre-harvest interval (PHI), i.e. the minimum interval between treatment and harvest.



#### WARNING !

Applying plant protection products may only be performed by a qualified person with adequate personal protective equipment (PPE), i.e. as recommended on the label, Material Safety Data Sheet (MSDS) or Information Sheet.

This may include: pesticide-proof mask, coveralls, long trousers, long-sleeved shirt, gloves and shoes ... [1].

# → All applications of plant protection products (including homemade preparations) must be documented in the batch and cultivation record!

# 2. Practical advice

Numerous expert guides propose natural pest control methods.

Some plants such as neem are particularly useful to include in a medicinal garden with Artemisia. For more information on this subject, see the AGRISUD Guide [2] p 149 to 154.

A field that supports biodiversity has a real beneficial effect on crops by controlling the overall impact of pests.

Planting Artemisia annua and Artemisia afra in new growing areas leads to the appearance of all kinds of attacks that are still unknown or not very well documented in the literature.

Below is a non-exhaustive list of pests observed on Artemisia in the Houses of Artemisia network together with feedback on tested control methods.

#### Butterfly larvae (chafer worms) in seedbed

These white worms devour roots and thus halt the growth of plants.

It is important to sterilise the soil used for sowing and protect the seedbed as indicated in File - Sowing.

Reducing watering and aerating the soil for new seedbeds has helped to combat the problem in Gabon.

#### Slugs (or other molluscs)



Artemisia annua seedlings and young plants are sometimes ravaged by molluscs (snails, slugs etc.).

Physical protection, such as a mosquito net, is effective.

Some members of the network use ashes, coffee grounds or salt powder to build a natural firewall.

Building a slug trap with beer is very simple and effective. Take a bowl and dig a hole. Place the bowl so that the rim is flush with the earth and fill with beer. Slugs love the smell, dive in and drown. If it rains, cover the trap (see diagram).

#### Wasps

Wasps have been reported to cut Artemisia leaves in the seedbed. Physical protection and using neem is said to be effective.

# **Defoliating caterpillars**

Some caterpillars can impact considerably on the growth of young Artemisia seedlings.

In Cameroon, a simple maceration of neem leaves (left to steep 24 hours in water) sprayed on young seedlings stopped caterpillar attacks.







#### Termites

Artemisia annua et afra are sometimes attacked by termites that gnaw at the base of the plant and cause it to die quickly.

Often, the impact across the whole plot is not so alarming. Especially if only a base branch is attacked (see photo).

To prevent this, it is important to avoid using wood (eg. for marking transplanting) or certain mulching matter that could attract termites.

If damage is significant, burying pieces of wood in the ground away from crops seems to keep termites away from Artemisia. Adding ash to sowing soil instead of sand, to the base of plants or in the bottom of transplanting holes can all be very effective. We have also heard of the beneficial effect of hot peppers, garlic and mint.

The use of neem leaves or cake in mulch is also interesting [2].

A row of vetiver (*Vetiveria zizanoides*) can also be interposed between rows of Artemisia. This grass has a repellent effect against ants and termites.

#### Ants

In many places, ants can be a problem at the sowing or cultivation site.

According to feedback we have received, neem is very effective in the fight against ants.

Homemade neem extract is very easy to make provided one has access to leaves of the tree:

- Crush 3 kg of leaves with a mortar.
- Soak in 10 litres of water for 6 to 12 hours until the water turns greenish.
- Strain.
- Add soapy water to bring the mixture up to 30 litres.
- Spray the mixture of macerated neem + soapy water at a rate of 3 litres per 10 m<sup>2</sup>.







- Repeat after 10 days if necessary.

It is also possible to buy <u>neem oil</u> commercially:

- Follow instructions to dilute in hot water.
- A small amount of soap can be added (5g for about 10 litres).
- Shake well to obtain a uniform mixture.
- Spray directly and shake from time to time.

# IMPORTANT: The IER (Rural Economy Institute) in Mali noted a phytotoxic effect of neem oil on young Artemisia annua seedlings.

Neem is an effective insecticide against many insects: caterpillars, aphids, beetle larvae, leaf miner flies, leafhoppers etc. [2]

Wood ash can be used against ants, aphids and flies:

Mix ½ cup ashes + ½ cup lime + 4 L water. Leave to stand for a while, filter and spray on plants.

Another suggested method is to mix 1 tablespoon of ashes with 1 L water and leave overnight. Then filter, add one cup fermented milk per litre, dilute the mixture in 3 times its volume of water and spray on plants.

NB: It is always advisable to check the efficacy or toxicity of the mixture on a few plants before treating all affected plants. [5]

As a preventive measure, unmacerated neem leaves or neem cakes can be used directly to combat insect infestation in the soil. Add to soil or mulch, use as green manure in seedbeds or mix into compost. [2]

A row of vetiver (Vetiveria zizanoides) can also be planted between rows of Artemisia.

This grass has a repellent effect against ants and termites.

Numerous crop associations have been proven effective against insects (pepper, tobacco, garlic etc.).

#### Nematodes

Irreversible wilting and plant death can be caused by nematodes, a type of worm found in the soil. These worms can be identified by uprooting dead plants to check for blisters on the roots.

If the impact is significant, treatment with neem or Tithonia can be useful (see Practical Guide to Agroecological Market Gardening in Brazzaville [4]).

A garlic solution (3 crushed cloves of garlic in 1 L water) has been shown to be effective at a dosage of 10 ml per Artemisia plant. Reapply once a fortnight for 2 months.

Also consider crop rotations and nematicidal plants: <u>http://ephytia.inra.fr/fr/C/</u> 20121/Hypp-encyclopedie-en-protection-des-plantes-Les-plantes-nematicides

#### Aphids

In Côte d'Ivoire, aphids have been found on *Artemisia afra* stems (see photo). They are responsible for the transmission of viral diseases to plants.

It has been observed that leaves crinkle, curl and narrow at the base (see photo).

Neem oil mixed with black soap has proven to be effective. It should be noted, however, that the IER (Rural Economy Institute) in Mali reported that neem oil was phytotoxic to young *Artemisia annua* seedlings.

See above for an ash-based preparation against ants, aphids and flies [5].

Artemisia annua is also attacked by aphids. The same treatments are recommended.







#### Woolly aphids

Woolly aphids have previously been observed on Artemisia annua.

This parasitic insect is characterised by its small size and a whitish waxy coating. On observation, it looks like it is covered in cotton down (do not confuse with mealy bugs, see below).

Leaves curl and stems are covered with a woolly white felt. Once the plant is impacted, growth is stunted and fungus and diseases can appear.

Start treatment as soon as woolly aphids appear as they multiply extremely quickly (a female can lay up to 100 larvae and generate 10 generations in 6 months).

A black soap-based preparation will eliminate woolly aphids. Mix 150 g of black soap with 1.5 L methylated spirits (household cleaning alcohol) and 10 L water and spray the plants.

Sow nasturtiums around the plants as a preventive measure.

#### Seedbed leaf miner flies

A case of leaf miner fly on *Artemisia annua* seedlings has been reported to us. Caterpillars dig galleries by eating the leaves, causing white lines to appear on the surface (see photo).

After removing affected leaves to limit the infestation, it may help to apply a garlic maceration. See above for an ash-based preparation against ants, aphids and flies [5].

Placing a protective cover over seedbeds can help if attacks are recurrent.







#### Mealybugs



Several cases of mealybug damage have been observed on Artemisia annua.

In Senegal, wilting of adult plants was the first visible symptom. Pink mealybugs, white wax and ants were observed on the roots. Mealy bugs form colonies just beneath ground level and ants feed on the honeydew in exchange for protection.

As the impact was minimal, removing affected plants from the field and burning them was sufficient.

In more severe cases, a preparation based on neem, tobacco or Tithonia can be effective. See the Practical Guide of the Agroecological Market Gardening in Brazzaville [4].

In several countries, white mealy bugs have been observed on the aerial part of Artemisia annua.



In Congo Brazzaville, generous watering of foliage helped to get rid of them. However, this method is not always effective in getting rid of eggs and larvae attached to the underside of leaves.

In severe cases, neem, tobacco or Tithonia preparations can be effective. (See the Practical Guide of the Agroecological Market Gardening in Brazzaville [4]).

The IER (Rural Economy Institute) in Mali reported that neem oil was phytotoxic to young *Artemisia annua* seedlings.



Alternative solutions to try are: one teaspoon of liquid black soap in 1.5 L of water or equal proportions of soap and oil diluted at a ratio of one teaspoon (= 5 ml) to 150 ml of water (source: S. K. K. (https://jardinage.ooreka.fr/fiche/voir/267948/lutter-contre-les-cochenilles)

Ashes, basil, garlic and nettles can be used for prevention. See the Practical Guide of the Agroecological Market Gardening in Brazzaville [4].





#### Leafhoppers (Spittlebugs)

At the larval stage, this leafhopper produces white foam as a defence system against pests. It sucks the sap from the plant.

According to feedback from our network, Artemisia plants are not overly affected. There is no need to worry too much because only the leaf on which the larva is laid is damaged and this can easily be removed by hand.

#### Locusts

These insect pests appear seasonally and eat Artemisia plants at any stage but seedlings and seedbeds are more vulnerable.

Neem leaf powder (dried and ground leaves) has shown a conclusive repellent effect in Côte d'Ivoire.

Songhai Centre in Porto Novo reported an effective method of treatment for locusts in Togo and Benin (particularly stinking locusts). Collect 10 locusts, crush and mix into 5 L water, then spray the solution on the plants.

Chickens and ducks are formidable predators for locusts but they should not eat Artemisia! Any poultry that does not eat vegetables and prefers insects to grass is a useful control method.



#### Crickets



In Côte d'Ivoire and Congo Brazzaville, crickets are said to cut branches off adult plants (*Artemisia annua* and *afra*) and bury them in the ground. Attacks are reported to be particularly frequent at night during the dry season.

However, the impact has not been significant enough for this problem to be addressed within the Houses of Artemisia network.

Nonetheless, if there is sufficient cause for concern, the simplest control method is to catch the crickets by hand or to set traps. A shallow bowl half-filled with water and a few spoonfuls of molasses can be used to bait them. In dry weather, another method is to dig a small furrow between infested crops, water abundantly and cover with a board. Crickets sheltering in the furrow can then be collected once or twice a day.

Diatomaceous earth and certain crop associations (coriander, beans, pulses, cloves, garlic and peas) can also be effective.

Note that the Food and Agriculture Organisation of the United Nations (FAO) considers that consuming this insect would be a great way to fight malnutrition since it provides high quality protein.

#### Goats and other wandering animals

Damage to Artemisia can be of great concern because the stems are often eaten directly with the leaves. The entire plant can be affected if it is still young. Fortunately, plants are very resistant to cutting and their growth is not too impacted if the central stem remains intact and attacks are not repeated.

The most effective means of protection against damage from wandering animals is to fence off the cultivation site with high, compact barriers to prevent animals from entering.

#### Rabbits

These rodents cut leaves, or sometimes whole stems of *Artemisia annua*, and leave them on the ground. They prefer tender shoots, which explains why attacks only occur in the first month after transplanting.

Damage to young seedlings is a concern since it hinders plant growth when the central stem is cut. However, the impact is minor when only a few leaves or secondary stems are cut. This is due to the ability of Artemisia to grow better after cutting.



Crop diversification is a way to keep rabbits away from Artemisia which they eat as a last resort.

If damage is significant, using a shotgun seems to be the most effective method of pest control. Unlike rats, rabbits are too wary to enter a cage, no matter what bait is placed.

Placing blue rags soaked in gasoline or hanging noiseproducing cassette wire on the windward side does not appear to scare them away. Carefully camouflaged traps placed in the impacted area have little effect.



Birds

Seedbeds and freshly transplanted seedlings are sometimes attacked by birds. They can be protected by covering with branches.

Virus

Although it has not yet been observed in the network, we have learnt of an emerging virus threatening *Artemisia annua*. This virus with spherical particles is said to cause plants to dry out and stunt growth. (<u>https://www.admin.ch/gov/fr/accueil/documentation/communiques.msg-id</u> 27951.html?fbclid=IwAR0tFpV6Ew5HzQ1KjiSpPr9pVr4YCQU2bWayu5lz16EfXXvzhYOAbttDxl0)

#### Damping off



If the seedbed is over-watered, seedlings can be attacked just before or a few days after germination by pathogenic fungi that cause damping off. If they have sprouted, the seedlings are weakened, soften and die quickly.

Conditions favourable to this disease are humidity, cold (T<10°C) and stagnant water.

Sterilisation of the sowing soil and careful watering to ensure soil is moist but not soaked help to avoid this fungal disease.

#### Pathogenic fungi of the genus Rhizoctonia

A pathogenic fungus of the genus Rhizoctonia was noted in several countries (Senegal, Benin etc.) in 2017. It is of concern, especially when plants are grown in high density and in a humid atmosphere.



The first symptoms of this fungal disease on Artemisia annua and afra are yellowing and/or browning of leaf tips followed by browning of stems at the base. Thereafter, a black line going up the central stem eventually extends to the whole plant and leaves become necrotic (die).

When *Rhizoctonia* spp. is present in the environment, it is important to control the water supply to avoid the presence of stagnant water at the roots. Mulching and drainage channels are useful to ensure that the soil is not too wet on the surface.

As a preventive measure, ensure there is sufficient aeration between plants to avoid humidity which is conducive to development of the fungi.



Remove all parts of plants (eg. blackened main stem), or entire plants if severely impacted, from the field as soon as the fungi is identified. Do likewise for any other affected plants around the growing site. Burn all contaminated plants. Do not compost. Repeat this procedure every day to limit the spread!

Cutting can promote healthy regeneration of foliage.

*Rhizoctonia* spp. is easily introduced into the crop by manual cultivation practices, unsterilised tools or pots, or via transplants with contaminated soil. Therefore, care should be taken to sterilise seedbed soil and to disinfect tools that come into contact with contaminated plants or soil.

If the spread of the disease becomes a concern, there are many natural antifungal recipes which can be used immediately after removal of any affected parts or plants.

In Cameroon, a preparation based on wood ash has been proven effective. Mix well 1 L wood ash with 10 to 15 L water, filter and sprinkle on diseased plants using a watering can or a spray.

This treatment has been shown to be even more effective in combination with a papaya and basil based preparation. Garlic or nettle liquid manure also works against soil-borne pathogenic fungi. For full recipes see the Practical Guide of the Agroecological Market Gardening in Brazzaville [4].

In Ghana, a 2-week application of a lemon, neem oil and ash solution was effective against Rhizoctonia spp.

Organically approved fungicide can be used if necessary. Treatment with a formula based on the antagonistic fungus of the genus Trichoderma is an option, but has not been tried by the Houses of Artemisia network.

This pathogen is persistent in the upper part of the soil. Following a major attack, it is useful to sterilise the soil or change cultivation site for the following year. To sterilise the soil, hoe then moisten the soil surface well, cover with a black tarpaulin and leave for at least 1 month in the sun.





#### **Powdery mildew**

This pathogenic fungus has been recorded on Artemisia annua in Rwanda.

A white colour slowly develops, forming a sort of white powder on the leaves which then spreads to the whole plant within a few days.

As with most fungi, the cause seems to be excess water that should be dealt with as soon as possible (see above for recommendations against the pathogenic fungus of the genus Rhizoctonia).

Treatment with 1-4% bicarbonate of soda (10-40 g of bicarbonate in 1 L water) seems to work. Spray affected plants once a week until symptoms disappear.

#### « Scorched » tips

This is due to excess fertiliser which has "scorched" the Artemisia.

IMPORTANT : Use only mature compost. Do not use uncomposted chicken manure !

#### « Burns » on lower branches of Artemisia afra

This is simply due to ageing of the stems (leaf senescence).

The lower leaves of Artemisia afra tend to become necrotic, in same way as for Artemisia annua.

The dried ("burnt") leaves on the stem are dead, without active molecules and cannot be used for herbal tea. Since leaves lose their beneficial properties as they age, we recommend harvesting lower leaves of plants before they turn yellow.

According to feedback from our network, this phenomenon is accentuated when the plant is not regularly pruned.







If the ground is wet, check that the roots do not turn black. This could point to possible attack by the fungus *Rhizoctonia* spp (see above).





In Gabon, removing and burning all dried leaves and pruning affected *Artemisia afra* plants has proven extremely effective (see File - Harvesting *Artemisia afa*).

Pruning rejuvenated plants, produced new leaves full of beneficial properties at the base and stimulated growth. (photos on the right).



# Early flowering

Pest attacks can trigger the appearance of flower buds, signifying the end of the vegetative phase and thus halting growth of Artemisia. If only one part of the plant goes into flower, removing it can allow the plant to resume growth if the source of stress is controlled.

To prevent early flowering, it is important to protect plants from any source of stress (water, wind, etc.). To protect from wind, consider wind-breaking trees and hedges.



**Plant malformation** 

Sometimes, a few Artemisia annua plants may exhibit abnormal appearance and growth.

For example, they can be much smaller and less productive than normal because their central stem thickens and twists (see photo on the right).

This phenotype may be the result of an undetermined external factor. However, it is probably simply the result of the significant genetic variability of the seeds distributed within the Houses of Artemisia network. This variability is of interest since it is the basis for adaptation of Artemisia to different growing conditions.

Collecting seeds from the most adapted and productive plants should be enough to overcome this problem by mass selection.

#### Albino seedlings

Sometimes Artemisia seedlings are completely white at the 2 cotyledon (false leaf) stage in the seedbed. This seems to be caused by a mutation or an albino gene due to genetic variability.

After 1 or 2 weeks, white seedlings die. They stop growing and gradually degenerate since they cannot photosynthesise.







Protective structures against wind, rain or direct sun are sometimes necessary.



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# Propagation



There are several methods to propagate plants. This important step requires care and rigour.

• Sexual reproduction

Seed production is always to be preferred over other methods because it enables plants to be adapted to local conditions.

Seeds supplied by the Houses of Artemisia network always show some variability. The genetic and phenotypic diversity of these seeds gives a great heterogeneity of plants in the field: depending on their genes and the environment, some plants will be more suitable than others. **Only seeds from plants adapted to local growing conditions** should be harvested in order to obtain plants that are better suited to these conditions. The aim is to allow each House of Artemisia to select one or more varieties<sup>1</sup> adapted to its environment and to become autonomous in seed production.

In spite of the important diversity of seeds and growing conditions within the network, no reports of inefficacy have been reported since 2012.

• Asexual reproduction

**Cuttings** and **layering** only propagate **clones**<sup>2</sup>. These methods are to be preferred when seed propagation is difficult. These two techniques therefore mainly concern the multiplication of *Artemisia afra*, whose seed viability is extremely low within the network.

Layering has a better success rate but depends on the number of stems that fall or can be bent to the ground.

Plants grown from cuttings do not have a good root system and are therefore more vulnerable to wind and drought in the first year.

<sup>&</sup>lt;sup>1</sup> Variety: sub-classification within the same species.

Remember, Artemisia annua and Artemisia afra are two different species of the genus Artemisia. There are therefore subcategories of Artemisia annua, which have different characteristics due to their different genetic material.

<sup>&</sup>lt;sup>2</sup> A plant clone is an individual or a group of individuals from a single individual ("mother plant") made by vegetative propagation, therefore not by sexual means: the processes of cuttings, layering, splitting clumps, grafting, in vitro cell multiplication produce clones.

Definition by Etienne Cuenot, Synthesis Tela Botanica network: <u>https://www.tela-botanica.org/wp-content/uploads/2017/11/clone.pdf</u>



# File 10 - Seed Production

Artemisia annua and Artemisia afra seeds are very small ovoid achenes<sup>3</sup>. The weight of 1000 seeds is about 0.1g.

**Under African conditions, the rate of flower fertilisation is generally very low**. This results in the production of a very small number of seeds per flower head. The seeds supplied by the Houses of Artemisia network are produced by sieving the contents of dried flower heads after flowering. They generally contain many impurities composed mainly of aborted florets (unfertilised flowers).

The number of fertile seeds per gram of these self-produced "seeds" generally varies between 100 and 300 but may be lower in some cases.

It is estimated that a good seed plant yields 25 g of these self-produced "seeds", giving between 2,500 and 7,500 fertile seeds.

The germination rate is extremely variable depending on the origin of the seeds, storage conditions and the local environment.

La Maison de l'Artemisia provides seeds from organic farming and asks that this method of cultivation is respected.

# **1.** Selection of seed plants

It is necessary to reserve seed plants for subsequent seed harvesting.

# Which plants to choose :

- I. The most attractive and productive plants to ensure maximum production of quality herbal tea per plant.
- II. Plants that are less vulnerable to pest and diseases.

**Choice of a resistant cultivar = the most effective means of control and always to be preferred**, even if yield is a slightly lower than with another non-resistant plant, because this avoids considerable production and financial losses when pests and disease appear again.

III. Plants located close to each other to allow for cross-fertilisation
 Artemisia annua plants are essentially self-incompatible in terms of fertilisation. Therefore, at least two adjacent seed plants are required for good seed production.

<sup>&</sup>lt;sup>3</sup> Achene : Dry one-seeded fruit that does not open to release the seed

- Mark seed plants before pruning and/or harvesting (e.g. with a ribbon).
- Do not harvest the leaves of Artemisia annua seed plants as this may limit the amount of seeds produced.
- Reduce watering by half after flowering to encourage seed production.
- Keep seed plants sheltered from wind to prevent seeds flying away.
- Leave the plants standing until the leaves dry and the whole plant turns brown (1 or 2 months) to allow the seeds to form properly and have time to ripen.

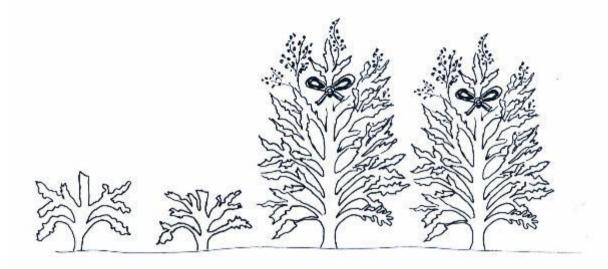


Figure 1 :Artemisia annua seed plant marked with a ribbon and not harvested for stems and leaves (Agathe Cornet-Vernet)



# 2. Seed collection



IMPORTANT : Do not harvest the seeds too early (they must have time mature correctly) or too late (seeds will fall to the ground and be lost).

- Test the maturity of seeds: if they are well formed, they fall when the branch is very lightly tapped.
- Cut mature Artemisia annua seed plants at the base OR remove mature seed-bearing branches of Artemisia afra.
- Dry on a clean tarpaulin or sheet and protect from moisture if the weather is humid.
- Tap the branches with a clean, dry stick to release the contents of the flower heads (seeds + flower pieces). OR shake over a basin, tarpaulin, sheet or clean, dry plastic cover.
- Sift the crop to remove as many impurities as possible. You can use a kitchen strainer and then a coarse-mesh sieve.
  - Seeds can be winnowed to ensure that they are very clean.
  - NB: this is the same process as for market garden plants of the Asteraceae family.

# IMPORTANT: Never use leaves from seed plants for herbal tea as they no longer contain active molecules!

# 3. Seed storage

Store seeds away from light, moisture and heat in a tightly sealed plastic or glass bottle.

Drying systems can be used, such as silica gel, rice or charcoal.

The pot of seeds can be stored in the ground to keep seeds cool and protected from light.

# WARNING: Never put self-produced seeds in the refrigerator as this will drastically reduce germination!

Seeds can be stored for up to 3 years at room temperature, but seeds produced under African conditions generally lose their germinative power after one year.



# File 11 - Propagation by cutting

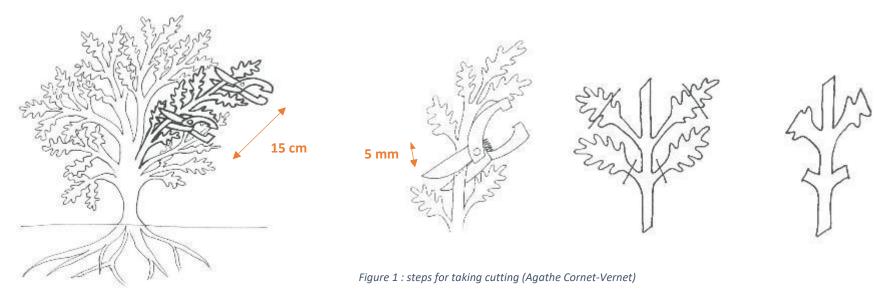
To be successful, this multiplication technique should be carried out during the rainy season or when the air is very humid.

Selection of plants for propagation: save the nicest and most productive plants; do not use plants that are prone to pests and disease.

#### How to take a cutting:

- Choose a long stem.
- Remove the tender, green tip growth and reserve for drying.
- Cut a 15cm length of semi-ligneous stem (one hand's length, semi-woody stem, starting to form ridges and turn brown) using a sloping cut 5mm under a leaf node (point where leaf is attached to stem).
- Remove lower leaves from cutting.
- Cut the top 2 leaves in half.

IMPORTANT : all cuts must be made with a sharp, clean, disinfected and dry tool!



#### **Rooting:**



- Plant the cutting 10 cm deep into the ground or a pot (use same soil mix as for seedbed) **OR** in a glass of water.
- Cover with transparent plastic sheeting, plastic bottle, cloche or frame in the dry season to maintain a warm and humid atmosphere.
- Keep out of direct sunlight for the first 4 weeks.
- Water cuttings and spray leaves with water every day to keep moist. Adjust watering according to rainfall and to avoid rotting.

Organic hormone rooting powder can be used to accelerate root development and increase success rate. A cold decoction of willow leaves over several days releases salicylic acid which can have the same properties as IBA indole butyric acid.

1 Artemisia afra plant = 250 cuttings / year!

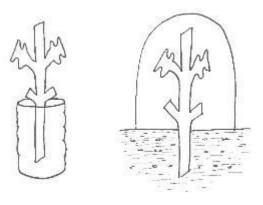


Figure 2 : cutting planted in small pot and open ground (Agathe Cornet-Vernet)

# File 12 - Propagation by layering

This propagation technique is to be carried out with Artemisia afra. It is not particularly useful for Artemisia annua which is an annual plant.

Layered shoots are encouraged to form roots white still attached to the parent plant.

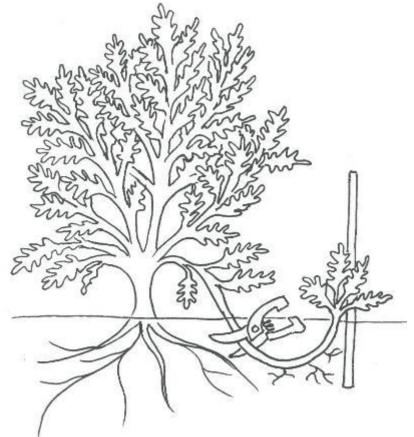
Selection of plants for propagation: use the nicest and most productive plants; do not use plants that are prone to pests and disease.

#### How to layer plants:

- Choose a flexible young stem that can be bent down to ground level (top stems can be used if long enough).
- Remove leaves from the area of the stem to be planted so as to promote root development.
- Clear any mulch, make a shallow trench a few centimetres deep, cover the stem with soil and firm in, leaving the shoot tip to point up.
- Secure the buried part of the stem in place with a small arch or two pieces of wood planted crosswise in the ground.
- Stake the shoot tip so it grows upwards.
- Water regularly around the buried part of the stem.
- Wait one to one and a half months.

#### Transplanting the layer:

- Remove the pieces of wood.
- Gently dig to uncover the buried stem and check for presence of roots.
- Cut the buried stem to separate the new plant with its new roots from the parent plant.
- Remove the new plant taking as much soil as possible around its roots to promote regrowth.



Artemisia

Figure 1 : Artemisia afra layering (Agathe Cornet-Vernet)



- Transplant at least 2 m away from the parent plant in watered, compost-enriched soil. (See: Cultivation Manual, transplantation).
- Water abundantly every day to keep plants moist. Adjust watering during the rainy season.

It is also possible to layer the stem directly in a pot with soil; this is a highly practical and effective technique if you want to have plants to give in pots.

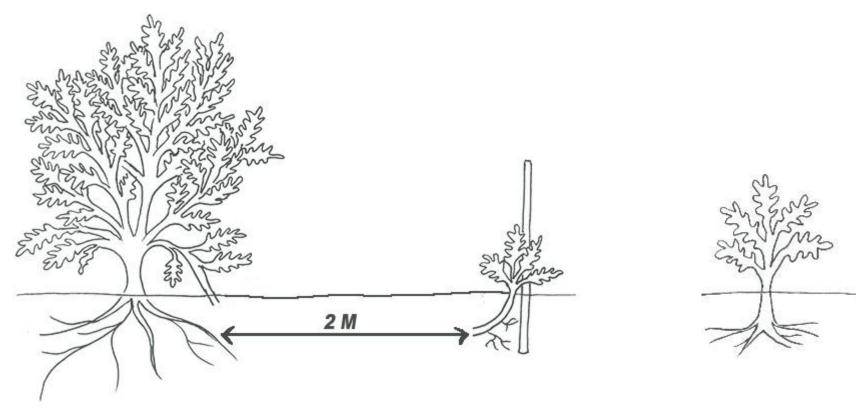


Figure 2 : separation of the plant obtained by layering (Agathe Cornet-Vernet)

# Processing



These operating procedures are standardised to ensure the quality of the end product.

We recommend herbal tea which is sterilised by boiling water at 100°C. Grind and sieve herbal tea before mixing into food, for example porridge for small children, so that it is absorbed easily.

> Powder is to be used as a second choice due to the risk of contamination. Moreover, powder oxidises quickly and has a much shorter shelf life.

# File 13 - Drying

Reducing water content limits damage caused by mould and other microbial agents and thus ensures long shelf life of the product.

#### Location :

WHO notes that harvested material should be dried immediately or as soon as practical.

The drying area should be protected from rain, insects, rodents, birds and other pests, livestock and domestic animals [1].

The drying area should be well ventilated and free of dust and other contaminants.

If the drying place is not close to the cultivation site, the harvest should be unpacked directly upon arrival.

IMPORTANT: Drying directly on the ground should be avoided!

If there is a concrete or cement floor, the medicinal plant material should be laid out on a clean tarpaulin, sheet or other clean piece of cloth.



### Method :

WHO notes that drying method and temperature can have considerable influence on the quality of the resulting medicinal plant materials [1].

# IMPORTANT! it is very important to <u>harvest on a dry, sunny day</u> and avoid harvesting in the rainy season. <u>The first days of drying in dry, sunny weather</u> will ensure the green colour and strong odour of the Artemisia.

#### Unlike most medicinal plants, drying in direct sunlight does not affect the therapeutic properties of Artemisia.

On the contrary, studies show that drying in the sun is more effective than freezing, drying in the oven or in the shade, because:

- It allows the bioconversion of artemisinin (43% for oven- and shade-dried plants compared to 94% for sun-dried plants) [2].
- It increases the concentration of active molecules against malaria (catechins, flavonoids, polyphenols, scopoletin, coumarins, etc.) [4-8].
- It reduces the antioxidant power of the plant, which is desirable for treating malaria [2-3].
- It destroys harmful molecules such as vitamin C, which inhibit the destruction of plasmodium by oxidation.
- Sun kills bacteria on the leaves and slows down mould (which, like any anaerobic composting, destroys many useful molecules and introduces potentially toxic fungi).

However, plants should not be left in full sunlight for too long to avoid deterioration of the foliage and loss of medicinal substances. Therefore, limit the time needed for optimal drying (see below, test for branches that break cleanly when bent at right angles).

Drying indoors will, however, limit exposure to dust if dust levels are too high.

Drying conditions should be noted in the batch and cultivation record.

It is important not to harvest everything in one go but to rotate the drying process.

According to our estimates, 200 m<sup>2</sup> of drying area can be used to dry a quarter of a hectare of crop (5,000 plants), rotating the drying process.

# Different cutting methods for drying :

# A. Drying whole plants

- Spread the plants side by side on a tarpaulin or dry and clean mat, in the sun or under shelter.
   OR Hang the plants upside down under a shed roof or on a line out in the sun or under shelter.
   Aerial parts of plants can also be arranged in bunches for hanging if care is taken to ensure that air can circulate through them. This is to ensure that they dry well indoors.
- 2. If plants dry outside, cover them at night to protect from animals and dew.
- 3. **Turn** the plants once a day to ensure uniform drying, i.e. to avoid scorching (if sunlight and temperatures are too high) and decomposition (mould if humidity is too high) of the plant material.

# This method is less demanding in terms of materials and labour, is better at avoiding mould, but drying takes longer.

You can expect to hang 1 tonne of Artemisia on ropes in a 600 m<sup>2</sup> drying area outside.

# **B.** Drying in segments

- 1. Cut the plants into segments of about 10 cm long.
- 2. Spread them in thin layers 15 cm thick at the most on a **tarpaulin** or dry and clean **mat**, in the sun or under shelter.

**Drying tables** or **racks** can be constructed. They allow for more uniform and faster drying thanks to ventilation from below and make it possible to work in an upright position. Racks can be made of different materials, but very fine mesh or fabric is recommended. It is important to choose solid materials, which allow air to pass through but retain leaves which crumble into powder when drying.

Racks should be kept clean and in good condition [1].



Figure 1 : Artemisia drying on tarpaulin and trays (Agathe Cornet-Vernet)





Forced ventilation solar dryers can be used if the temperature is kept below 40°C.

This system appears to be fast and very effective in preserving the organoleptic characteristics of the plant (odour, flavour, colour). Moreover, it protects against contaminants (dust, bird excrement, insects, ...), allows ventilation and avoids night-time humidity.

It is particularly recommended to add mechanical ventilation if drying takes place indoors.

3. **Turn** the segments twice a day to ensure uniform drying, i.e. to avoid scorching (if sunlight and temperatures are too high) and decomposition (mould if humidity is too high) of the plant material.

### This method is more demanding in terms of materials and labour, but enables faster drying.

(3-4 days of sunshine rather than 4-5 weeks for whole plants!)

It shortens the drying cycle and hence the drying area required per cultivation area. It is very effective in dry climates.

# CAUTION: Avoid cutting plants directly into segments if the weather is wet, as mould will develop very quickly!

This method of drying directly in segments requires a 24-hour rain free period before harvesting and dry and sunny weather.

For **optimal drying in tropical climates** (such as southern Benin), harvesting is done in dry, sunny weather and plants are first dried whole for several days. When they are less moist and the weather is dry, they are then cut into segments to speed up the drying process and are dried in a few days on racks.

**IMPORTANT:** Drying is optimal when branches break cleanly when bent at a right angle! Plant material should be turned sufficiently so that it does not overheat. The temperature should ideally remain below 40°C, and definitely below 60°C to preserve volatile compounds and essential oils of the plants. [9] Phytosterols, saponins and fatty acids are not greatly affected by high drying temperatures. Only temperatures above 80°C show significant decreases. [10]

Dried crops should be packed in clean, dry bags as soon as possible to protect the product from deterioration and unnecessary exposure to possible pest attacks and other sources of contamination [1].

Refer to WHO recommendations for good storage practices.

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# File 14 - Herbal Tea Production

Hands must be thoroughly washed with soap before handling, all equipment and utensils must be clean and dry. Anyone handling plants must wear clean and dry gown, mask and gloves.

Refer to Personnel and Equipment sections of WHO Guidelines on Good Agricultural and Collection Practices (GACP) for medicinal plants for more details on hygiene standards and for the processing facility.

- Inspect the dried crop to remove blemished, mouldy and damaged plant material as well as soil particles, stones and other foreign matter [1]. There is a wide variation in stem (red, green and brown) and leaf (green and brown) colors that are not indicators of quality.
- Cut the leaves and stems with a machete or a conventional 16 or 24 hammer mill shredder to obtain 2 cm long segments.
   IMPORTANT: It is normal for the leaves to crumble into powder once dry, but do not cut the HERBAL TEA into sections of less than 1 cm! It will quickly turn into POWDER which oxidizes rapidly with beneficial properties lasting up to 6 months maximum. Herbal tea beneficial properties last for more than 3 years.

NB : Parts of the central stems that are too thick for shredding can be used as incense sticks with antiseptic, purifying and anti-mosquito properties.

- Store herbal tea in clean, dry and preferably new bags [1-2].
- To kill possible insect eggs, the herbal tea can be bulk stored in the freezer in large, clean, dry, airtight bags for 3 days or more.
   IMPORTANT: Use well-sealed and airtight bags so that any condensation only occurs outside the bag and does not re-moisten the Artemisia. For defrosting, simply take the bag out of the freezer and leave about 24 hours to thaw.
- Use paper bags or pre-printed 40 g envelopes with regulation label for packaging.
   IMPORTANT: Use plastic-free and food-grade kraft paper!

Packaging must meet WHO standards and be non-polluting, clean, dry, undamaged, tear-resistant and in conformity with standards required for the plant in question [2].

Reusable packaging materials should be thoroughly cleaned and dried before use.

Store in a clean, dry, pest free place that is protected from livestock, pets and other sources of contamination. [2]

#### **References :**



1. World Health Organisation. WHO guidelines on good agricultural and collection practices (GACP) for medicinal plants. 2003. Available at : <u>https://www.who.int/medicines/publications/traditional/gacp2004/en/</u>

2. World Health Organization. WHO monograph on good agricultural and collection practices (GACP) for Artemisia annua L. 2006. Available at: <u>http://www.who.int/malaria/publications/atoz/9241594438/en/</u>



# File 15 - Leaf and Stem Powder Production

IMPORTANT : Powder is extremely susceptible to contamination by mould and bacteria. It is highly sensitive to moisture and grinding into fine particles considerably increases penetration of micro-organisms and oxidation of the active molecules! We recommend that only Artemisia annua and Artemisia afra be used in herbal teas.

However, powder is useful and effective for the production of capsules and tablets, combination with food and even production of suppositories.

Before handling, hands must be washed thoroughly with soap, equipment and all utensils must be clean and dry, and **disinfected**. (White spirit vinegar can be used. 90° alcohol does not destroy everything and bleach is dangerous for health if it is not rinsed thoroughly! For a natural solution, use 5L of water with 10 ml of palmarosa essential oil, 10 ml of tea tree essential oil and 10 ml of grapefruit seed extract). Anyone handling plants must wear a clean and dry gown, mask and **disposable gloves**.

Refer to "Personnel" and "Equipment" sections of WHO "Guidelines on Good Agricultural and Collection Practices (GACP) for medicinal plants" for more details on hygiene standards and for the processing facility.

Particular care must be taken when processing into powder form to avoid any risk of contamination.

- Inspect the dried crop to remove blemished, mouldy and damaged plant material as well as soil particles, stones and other foreign matter [1]
   There is a wide variation in stem (red, green and brown) and leaf (green and brown) colours that are not indicators of quality.
   Grind leaves and stems with a pestle or with a conventional hammer mill with 16 or 24 hammers to obtain the finest possible segments.
   NB: Parts of the central stems that are too thick for grinding (1 inch thick) can be used as incense sticks with antiseptic, purifying and mosquito repellent properties.
- If necessary, store the ground material in hermetically sealed containers in a cool, ventilated and shaded location.
- Sieve with a fine sieve (maximum mesh size 2 mm) to obtain fine powder to fill capsules correctly or to mix into honey.
- Store the fine powder in hermetically sealed jars and in a cool, ventilated and shaded location.
- Place the jars of fine powder in the **freezer for 3 days** to ensure that any insect eggs are destroyed. Jars must be tightly closed and airtight so that any condensation only occurs on the outside and does not re-humidify the Artemisia.

To defrost, leave the jars out of the freezer for about 24 hours until the temperature is raised.

- LA MAISON DE CArtemisia
- Package the powder in clean, dry and preferably new small jars or sachets [1-2].
   IMPORTANT : Use kraft paper accepted for food hygiene or food grade plastic and close tightly with tape, knot or other reliable system.

**Capsules:** Capsules come in several sizes (#0 small, #00 medium and #000 large) and must be made of vegetable matter (without animal gelatine). They are bought empty, closed and supplied with a capsule filler.

#0 capsules contain about 0.25g of powder and are the easiest for children to swallow, #00 capsules contain about 0. g and #000 capsules contain 0.8 to 1g depending on the fineness of the powder and how compact it is.

To use a basic capsule filler, open the capsules, place the large part in the small holes of the filler, fill the capsules with powder (by pouring powder on the filler and scraping with a ruler), tamper twice to compact powder (with a clean nail, for example) and close the capsule with the small lid. Semi-automatic capsule fillers and machines are also available.

ALL materials used for packaging or coming into contact with the powder must be non-polluting, clean, dry and in good condition and comply with quality standards for the medicinal plant materials concerned [1].

Reusable packaging materials should be thoroughly cleaned and dried before reuse [1].

Store in a clean, dry and shaded place, free from pests, inaccessible to livestock, domestic animals and other sources of contamination! [2]

#### Bibliography :

1. World Health Organisation. WHO guidelines on good agricultural and collection practices (GACP) for medicinal plants 2003. Available at: <u>https://www.who.int/medicines/publications/traditional/gacp2004/en/</u>

2. World Health Organization. WHO monograph on good agricultural and collection practices (GACP) for *Artemisia annua* L. 2006. Available at: <u>http://www.who.int/malaria/publications/atoz/9241594438/en/</u>