Assessment of phenology and morphological diversity of 3 species of Asteraceae: *Anacyclus clavatus*, *Chamaemelum fuscatum* and *Tanacetum parthenium*

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Abstract

The present paper deals with three species of Asteraceae : *Anacyclus clavatus*, *Chamaemelum fuscatum* and *Tanacetum parthenium* that have a wide range of uses in medicine and in industry. The detailed morphological characterization and the phenology are discussed. These species were characterized by inter-specific variations using 18 morphological characters and the study of phenological activities like vegetatif study, flowering time, fruiting time and seed formation for two consecutive years from 2009 till 2010.

The results of phenological study show that the 3 species studied have distinct phenologies. The longest phenological cycle is observed for *Tanacetum parthenium.* The results of the variance analysis showed significant differences to highly significant for the majority of the traits studied. The comparison of means reveals that *Anacyclus clavatus* and *Chamaemelum fuscatum* form a single group for most of the traits measured, while *Tanacetum parthenium* is clearly distinct from these two species. In addition, the principal component analysis confirms the results of the variance analysis and the comparison of means. It showed that *Anacyclus clavatus* and *Chamaemelum fuscatum* are divided into two overlapping groups, the group where *Tanacetum parthenium* is located is quite distant.

***Keywords**: Anacyclus clavatus; Chamaemelum fuscatum; Tanacetum parthenium; morphological; phenology.*

1. Introduction

Phenological study is important in plant management and combating deforestation, honey analysis, floral biology, estimation of reproductivity and regeneration [1]. It is important also in understanding species interrelations and their interaction with the environment. Variations in phenophases among individuals of different species have been linked to environmental perturbations [2]. A clear understanding of phenological behavior on time of anthesis, time and duration of stigma receptivity, fertilization, mode of pollination, seed development is necessary for breeding programs to obtain better traits [3]. Thus plant phenological study has great significance because it not only provides knowledge about the plant growth pattern but it also provides the idea on the effect of environment and selective pressure on flowering and fruiting behavior [4].

Evaluation and characterization through morphological parameters of different crop germplasm is therefore so much important for all plant breeders [5]. Therefore, it is important to make proper strategies for the collection and evaluation of germplasm sources which are locally used in different regions of the world and save them from being vanished [6]. To have a variety of better traits of any crop we need information's about its genetic diversity [7]. Thus, characterization and estimation of genetic diversity is an important step for the competent and successful maintenance and utilization of different crop germplasm [8].

Genetic diversity is an inherited variation among and between populations, created, activated and maintained by evolution [9]. Morphological traits provide a simple way of measuring genetic diversity while studying genotype performance under normal growing conditions, but are influenced by environmental factors ([10]; [11]). Plants have the potential to response to the changed environments by changing their morphology and there for, the intra-specific variation in plant characteristics is usually regarded as the adaptive mechanism to different environments [12].

The Asteraceae is one of the largest families, comprising 250.000 species, It is known for its wide range of uses not only in medicine but also some plants are grown as ornamental plants such as chamomile (*Tanacetum parthenium*), others can provide different products: natural rubber, colorants, insecticides and spices [13].

A. clavatus (*Anacyclus clavatus* (Desf.) Pers.) is an annual self-incompatible herb, belonging to the Asteraceae family, is an herbaceous, annual and spontaneous plant that is found almost everywhere in the Mediterranean region [14]. It's 20 to 50 cm tall, hairy, green or whitish-green, with an upright or ascending stem, woolly and rowdy whose branches are divorced. Leaves are bipinnate, long to very narrow segments terminated by a small mucron [15]. The convex or somewhat conical receptacle carries triangular bracts, ovals in the shape of sequins. The inflorescences have two types of hermaphrodite flowers: the central flowers are yellow-colored and the peripheral flowers are tongued, long and white. They flourished from March to June [14]. The fruits in the form of akene are small, very compressed cuneiform and of grey to beige colour [15]. The number of chromosomes of this species is 2n = 18 [16]. It's a plant that grows on the edges of fields and roads and in the wastelands of the entire Mediterranean coast [15]. In Tunisia, it's is located in the north (Kroumirie, Oued Medjerda and Cap Bon), and in the center. The use of this species is very limited. The aerial part of *A.*

clavatus is used as a powder against stomach pain. It may also be one of the components of tobacco [17].

C. fuscatum (*Chamaemelum fuscatum* (Brot.) Vasc.), belonging to the Asteraceae family, anthemidae tribe, and Ormenis sub-section, is an annual, herbaceous, glabrous 30 cm rowing, ascending or upright. The leaves are bipinnate. The heads are heterogeneous with yellow disc and white ligules; their flowering is very early from November to April. The akene is very small, striated, tetragonal and brown to yellow in colour. It's a very widespread plant on the banks of the seguias.

In Tunisia, *C. fuscatum* is found in the north (Ain Drahim, Kef), in the center (Sousse, Enfidha) and in the South (Gabes). Internationally, It's located in the western Mediterranean basin of Spain, Greece and North Africa (Tunisia, Morocco and Algeria) [15]. The number of chromosomes of this species is 2n = 18 [18]. It's known for its anti-malaria property and its protective effect against cell damage [19].

L. parthenium (*Leucanthemum parthenium* (L.) Gren. & Godr) ou *Tanacetum parthenium* (L.) Schulz Bip. belongs to the Asteraceae family too, the Anthemidae tribe and the Asteroida subfamily [20]. This chamomile is a very fragrant, perennial, rooted plant, with flowering stem erect without hair. The leaves are deeply divided into 4 to 12 toothed segments. The internal tubular flowers are yellow and the ligulate external flowers are white. They flourish from June to August in European conditions [14] and from July to October in Iran [21]. The ripe fruits are brown, glandular and surmounted by a very short membranous crown.

Tanacetum parthenium (L.) Schulz Bip. is a medicinal plant used primarily for the prevention and reduction of migraine attacks frequency, against stomach aches and malaria [22]. It's also known for its properties: antiseptic, stomachic, antihysteric, vermifuge and insecticide. It's found spontaneously on the edges of roads and often in the vicinity of dwellings and it can also be grown in gardens as an ornamental plant. Internationally, *Tanacetum parthenium* (L.) Schulz Bip. is found almost all over Europe except the boreal zone and it is also found in South-Western Asia [14].

However, there is little information on the morphological diversity and the phenology of *Anacyclus clavatus* (Desf.) Pers., *Chamaemelum fuscatum* (Brot.) Vasc. and *Tanacetum parthenium* (L.) Schulz Bip. and the potential of these species in breeding programs. The aim of this study is to assess the variations in morphology and phenology of these 3 species.

2. Materials and methods

2.1. Plant material and experimental design

Three species of Asteraceae have been studied in this work: *Anacyclus clavatus*, *Chamaemelum fuscatum* and *Tanacetum parthenium*. These species were grown on an experimental plot at the Faculty of Sciences of Tunis, Tunisia under uncontrolled conditions. The seeds used originate from Esbikha for *A. clavatus*, Haouz (Morocco) for *C. fuscatum* whereas the seeds of *Tanacetum parthenium* are available in the laboratory of Genetics and Bioresources of the Faculty of Sciences of Tunis.

2.2. Phenological characters

Different phenological stages presented by the individuals of each species are defined:

2.2.1. Vegetative period

This stage spreads from the planting to the beginning of flowering. This is the phase of vegetative growth.

2.2.2. Flowering

This is the period during which the flowers appear. The method of study is based essentially on the visual observation of the appearance of the flowers.

2.2.3. Fruiting

This phase is characterized by the formation of the fruit. It begins with the formation of the first seeds and ends with the general ripening of the seeds.

2.3. Morphological traits

In order to compare the various species studied, we describe the characters of their vegetative part: The type of branching, the stem, the structure of the leaves, the structure of the inflorescences and flowers, the structure of akene and the weight of 100 akenes.

Measurements of the morphological characters were performed on three samples of *Anacyclus clavatus*, *Chamaemelum fuscatum* and *Tanacetum parthenium* grown in the Faculty of Sciences of Tunis, for each species, we have studied 10 individuals. The 18 morphological quantitative traits were assessed to characterize and estimate genetic diversity among the 3 species studied, the quantitative traits measured were:

- Length of main axis in cm: LAP
- Average length of primary branches in cm: LMRP
- Average length of branches in cm: LMRS
- Average length of the tertiary branches in cm: LMRT
- Length of main root in cm: LRP
- Number of leaves per plant: NF
- Average diameter of the receptacle in cm: DMR
- Average number of leaflets per leaf: NLL
- Average length of the leaf rachis in cm: LMRF
- Number of inflorescence per plant: NI
- Number of primary branches: NRP
- Number of secondary branches: NRS
- Number of tertiary branches: NRT
- Average number of ligules per head: NML
- Number of ligules of the main axis head: NLCAP
- Length of the smallest branch in cm: LPR
- Length of the longest branch in cm: LLR
- Weight of 100 akenes : P_{100} A

2.4. Data analysis

The evaluation of a collection of genetic resources is commonly based on the simultaneous examination of many populations for various morphological characters. In this context, data on the different morphological traits measured were:

• An analysis of variance with one classification criterion followed by a comparison of means.

• An estimate of the degrees of association between the different traits studied by the Pearson correlation coefficient [23].

• A principal component analysis (PCA) based on the derivation of orthogonal variables [24].

In order to evaluate morphological diversity and to establish relationships among studied species, several statistical procedures were conducted. Quantitative data were computed using the software XLSTAT version 2011 to perform analysis of variance, comparison of mean using the Duncan test and to calculate the Pearson correlation coefficient. Principal component analysis (PCA) was also done using the software XLSTAT.

3. Results and discussion

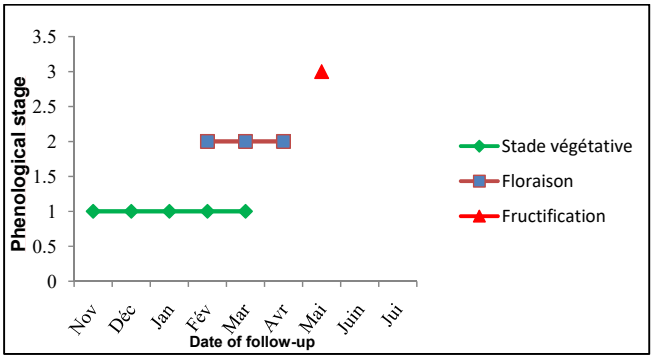
3.1. Phenology study

3.1.1. Vegetative period

The vegetative period is characterized by a strictly herbaceous development and extends from seedling to full bloom. We divided this phase into 2 stages:

Stage of germination: it is characterized by the appearance of the primordial leaves. In all three species, the germination begins after 10 days.

Stage of foliage: Observation of the phenological spectrum reveals that this stage is the longest of the phenological cycle. This stage, which is characterized by the growth of the stems in length and by the formation of the leaves, lasts 6 months for *Chamaemelum fuscatum* (Figure 1) and 7 months for *Anacyclus clavatus* (Figure 2). *Tanacetum parthenium* is a perennial herb plant (Figure 3).



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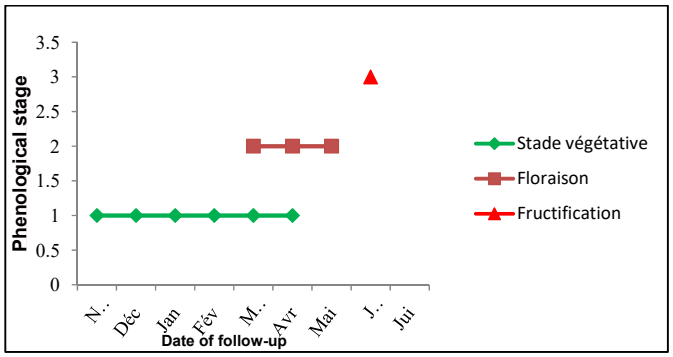
178 **Fig.1.** Different phenological phases of *Chamaemelum fuscatum*



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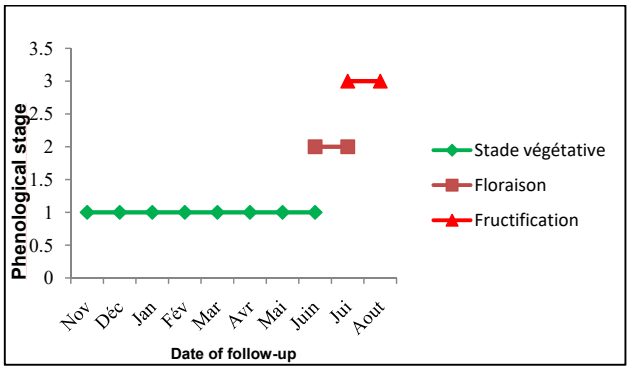
180 **Fig.2.** Different phenological phases of *Anacyclus clavatus*



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182 **Fig.3.** Different phenological phases of *Tanacetum parthenium*

3.1.2. Flowering

Flowering is considered from the formation of the first flower until most flowers have evolved this period differs from one species to another: For *Chamaemelum fuscatum*, the flowering period ranges from mid-February to the end of April (Figure 1). For *Anacyclus clavatus*, this period extends from the end of March to mid-May (Figure 2). For *Tanacetum parthenium*, the first flower blooms in early June and full bloom is observed around mid-July (Figure 3).

Flowering appears to be highly favoured during the rainy season for *Anacyclus clavatus* and *Chamaemelum fuscatum*, only *Tanacetum parthenium* flowers during the dry season. We find that the species *Chamaemelum fuscatum* characterized by a very early flowering date has a spread flowering period. In addition, the species *Tanacetum parthenium* characterized by a late flowering date has a relatively short flowering stage and this to escape the water stress.

3.1.3. Fruiting

It is the formation of fruit in the form of akene. We have noticed that the appearance of the first akene coincides with the peak of flowering, while the full fructification characterized for the 3 species by the change of color flowers in tubes from yellow to light grey and the fall of the white ligules is generally obtained after two weeks of the appearance of the first fruit (Figure 1, 2 and 3).

In fact, the study of [25] reveals that akenes of *A. clavatus* that germinated earlier produced plants with higher biomass and higher reproductive effort. In addition, this work show that the phenology of *Anacyclus clavatus* akene germination was the main factor affecting post dispersal life-history traits related to competitive ability and reproductive success.

In addition, the study of [26] showed a high phenological diversity for the four phenological patterns (buds, flowers, fruits and seeds) among fifteen leguminous plant species growing in Amritsar.

3.2. Morphology study

3.2.1. Study of vegetative part

A comparative morphological characteristics of the 3 species studied is shown in Table 1.

Table 1: Morphological characteristics of *Anacyclus clavatus*, *Chamaemelum fuscatum* and *Tanacetum parthenium*.

Species	NR	Leafs	Flowers	Akenes	P_{100} A in mg	DR (cm)
Anacyclus clavatus	T+5	Dark green bipinnate	White ligulated flowers	Beige	45.23	1.56 ± 0.01
Chamaemelum fuscatum	T+5	Green bipinnate	Flowers in yellow tubes	Brown to yellow	26.63	0.67 ± 0.05
Tanacetum parthenium	T+3	Greenish-yellowish divided into wide	White ligulated flowers	Brown	9.96	0.65 ± 0.02

NR: number of ramifications, **P_{100} A:** weight of 100 akenes, **T**: number of branches, **DR**: diameter of the receptacle.

The inflorescences and the flowers

The inflorescence of *Anacyclus clavatus*, *Chamaemelum fuscatum* and *Tanacetum parthenium* is a flower head containing two types of flowers: yellow flowers tubulated in the center and white flowers ligated at the periphery. The flowers of the 3 species have the same floral biology, but show a difference in floral structure. Indeed, the liguled flowers of *Chamaemelum fuscatum* are long and beaked at the tip, while those of two other species are similar; they are short and more or less rounded.

The diameter of the receptacle varies from one species to another. It is 0.65 ± 0.02 cm in *Tanacetum parthenium*, 0.67 ± 0.05 cm in *Chamaemelum fuscatum* and 1.56 ± 0.01 cm in *Anacyclus clavatus*.

Fruit

The fruits differ between the 3 species studied. The fruit of *Anacyclus clavatus* (Figure 4) is an indelible akene, beige at maturity, of rectilinear shape to flattened cone. This akene is surrounded by two membranous wings, clear, very thin, parchment and truncated at the apex. In the case of an akene without these wings, the fruit appears mottled and has four longitudinal ribs.

The fruit of *Chamaemelum fuscatum* (Figure 5) is an indehiscent akene, very small, not marginated, flattened ovoid, raised by 3 ribs weak and finely striated. Their color is brown to yellow at maturity. The fruit of *Tanacetum parthenium* (Figure 6) is an indehiscent akene, very small, brown at maturity, glandular and surmounted by a very short membranous crown and crenate.

Weight of 100 akenes

The mean weight of 100 akenes of *A. clavatus* is 45.23 mg. For *C. fuscatum*, it is 26.63 mg. An average weight of 9.96 mg was calculated in *Tanacetum parthenium* (Table 1).

3.2.2. Analysis of morphological variability

3.2.2.1. Analysis of variance

The analysis of variance with one classification criterion (species effect) showed highly significant differences between the three species studied (Table 2) for the majority of the quantitative traits measured such as: Length of the longest branch (LLR), Length of the smallest branch (LPR), number of secondary branches (NRS), number of primary branches (NRP), mean leaf spine length (LMRF), average number of leaflets (NLL), mean diameter of the receptacle (DMR), length of the main root (LRP), mean length of the tertiary branch (LMRT), average length of secondary branch (LMRS), average length of primary branch (LMRP) and length of the main axis (LAP). The difference between the three species is not significant for: The number of the principal axis head ligules (NLCAP), the average number of ligules per capitule (NML) and the number of tertiary branches (NRT). This result reflects a phenotypic heterogeneity between the 3 species studied, taking into account the measured parameters.

Table 2: Variance analysis of the morphological characters.

Characters	df	Average square	F_{obs}	Pr › F
LAP	2	3730,630	68,058	< 0,0001 **HS**
LMRP	2	982,641	26,382	< 0,0001 **HS**
LMRS	2	862,412	52,589	< 0,0001 **HS**
LMRT	2	360,894	26,359	< 0,0001 **HS**
LRP	2	40,961	11,73	0,000 **HS**
NF	2	338256,13	5,355	0,011 **S**
DMR	2	2,701	108,846	< 0,0001 **HS**
NLL	2	150,633	75,039	< 0,0001 **HS**
LMRF	2	11,796	36,769	< 0,0001 **HS**
NI	2	30601,433	2,983	0,068 **NS**
NRP	2	185,633	14,312	< 0,0001 **HS**
NRS	2	14770	15,244	< 0,0001 **HS**
NRT	2	4548,433	0,867	0,432 **NS**
NML	2	226,9	1,258	0,3 **NS**
NLCAP	2	0,7	1,086	0,352 **NS**
LPR	2	15,74	22,619	< 0,0001 **HS**

LLR	2	935,217	8,415	0,001	**HS**

df : degree of freedom; $\mathbf{F}_{obs}$: F observed ; **HS**: highly significant; **S**: significant ($P < 0.05$) ; **NS**: no significant ($P \geq 0.05$).

3.2.2.2. Comparison of means

According to the Duncan test, we distinguish 5 types of groups (Table 3). Comparison of means shows that *A. clavatus* and *C. fuscatum* are distinctly different from *Tanacetum parthenium* for: the length of the main axis (LAP), the mean length of the secondary branch (LMRP), the average length of the tertiary branch (LMRT), Root length (LR), number of leaves (NF), number of primary branches (NRP) and number of secondary branches (NRP).

A. clavatus is distinguished from *Tanacetum parthenium* and *C. fuscatum* for the mean diameter of the receptacle (DMR), the length of the smallest branch (LPR) and the length of the longest branch (LLR). In fact, the three species did not differ significantly in the mean diameter of the receptacle (DMR), the length of the smallest branch (LPR) and the length of the longest branch (LLR).

The parameters discriminating the three species are: the average length of the primary branch (LMRP), the mean number of leaflets per leaf (NMf) and the average length of the spine (LMRF). For the number of inflorescence per plant (NI), *Anacyclus clavatus* is not significantly different from *Chamaemelum fuscatum* or *Tanacetum parthenium*. Therefore, *Anacyclus clavatus* and *Chamaemelum fuscatum* are much alike for more than half the morphological characters studied. Most of the highest averages of the morphological traits are observed in *Anacyclus clavatus*, while the majority of the lowest averages are observed in *Tanacetum parthenium* (Table 3).

Table 3: The Duncan test of the 3 species studied.

Traits	*Anacyclus clavatus*	*Chamaemelum fuscatum*	*Tanacetum parthenium*
LAP	19,8 B	20,71 B	53,7 A
LMRS	20,6 A	17,91 A	3,39 B
LMRT	12,12 A	12,5 A	1,91 B
LR	8,1 B	7,72 B	11,4 A

NF	629,5 A	524,5 A	271,7 B
NRP	11,4 B	11,9 B	19,1 A
NRS	39,6 A	29,6 B	100,6 A
DMR	1,56 A	0,67 B	0,65 B
LPR	3,21 A	1,4 B	0,8 B
LLR	46,69 A	29,97 B	29,91 B
NRT	53,7 A	37,3 A	79,6 A
NML	11,7 A	19,9 A	11,6 A
NLACP	13,3 A	13,4 A	12,9A
LMRP	36,12 A	24,34 B	16,42 C
NMf	15,6 A	10,9 B	7,9 C
LMRF	4,36 A	3,19 B	2,19 C
NI	116,5 A and B	82,4 B	190,6 A

3.2.2.3. The Matrix of correlation coefficients

The matrix of correlation coefficients between the characters studied (Table 4) shows: A positive correlation of the following traits: LMRP and LMRS correlate positively with each other and with all the parameters of LMRT, NF, DMR, NLL, LPR and LLR ; The character LAP is strongly correlated positively with the parameters LR, LMRF, NI, NRP and NRT ; A highly significant positive correlation between LMRF with NI, NRP and NRS ; NI correlates strongly with the parameters : NRP, NRS and NRT and weakly with LLR ; NRP is strongly correlated with NRS and weakly correlated with the characters NRT and LPR. The LAP has a highly significant negative correlation with the parameters (LMRS, LMRT, NLL) and significant with the characters (LMRP, NF, DMR, LPR); LMRS. It is important to note that NLCAP and NML are not correlated with any of the other characters and that LMRP is the most positively correlated with the other traits (Table 4).

3.2.2.4. Principal component analysis

The graphical representation of the individuals dispersion of the 3 species studied reveals a homogeneous grouping of the species studied forming 3 clear groups (Figure 7).

Indeed, there is a slight overlap between the two groups: *Anacyclus clavatus* and *Chamaemelum fuscatum*, whereas, *Tanacetum parthenium* group seems very distinct from the two others species. These results confirm those of the variance analysis which showed a strong resemblance between *Anacyclus clavatus* and *Chamaemelum fuscatum*.

It is also observed that the individuals of the species *Chamaemelum fuscatum* occupy a rather restricted part of the plane and are located entirely in the negative part of the two axes F1 and F2. While, the individuals belonging to *Anacyclus clavatus* are scattered on the two axes (F1 and F2) with a trend towards the positive values of the F1 axis (Figure 7).

Furthermore, individuals of *Tanacetum parthenium* are the best dispersed on the 2 axes (F1 and F2) with a tendency towards the negative values of F1 axis (Figure 7).

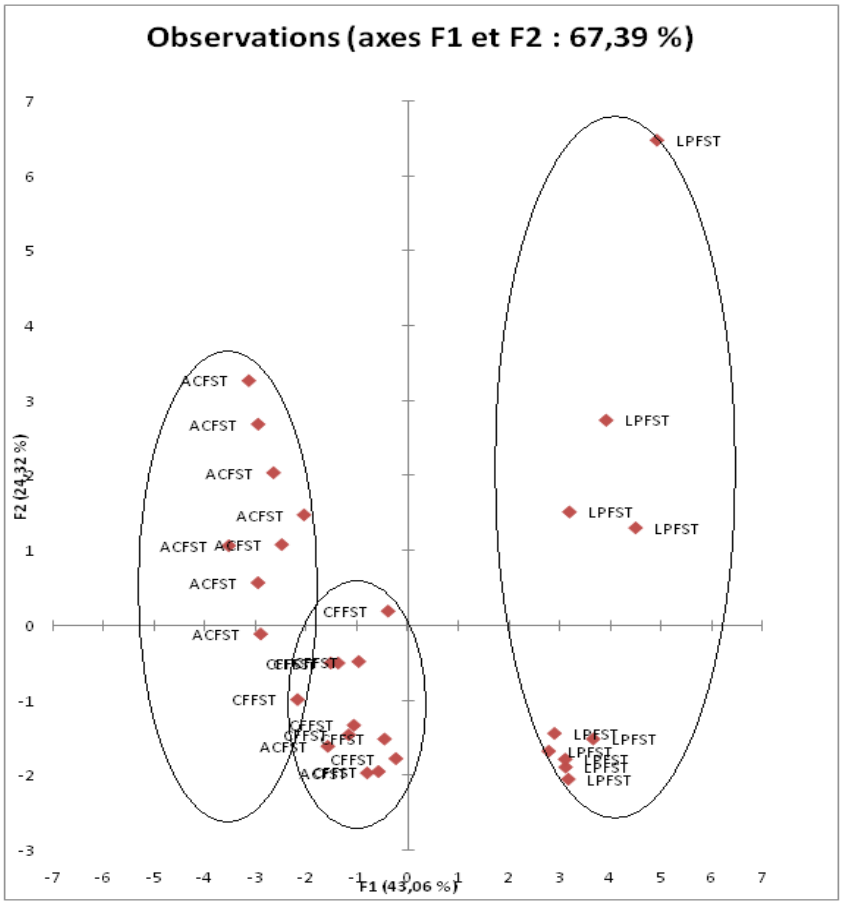

Fig.7. PCA analysis for *Anacyclus clavatus*, *Chamaemelum fuscatum* and *Tanacetum parthenium*

Table 4: Correlation of the morphological traits.

Traits	LAP	LMRP	LMRS	LMRT	LR	NF	DMR	NMf	LMRF	NI	NRP	NRS	NRT	NML	NLCA	LPR	LLR
LAP	1																
LMRP	-0,536	1															
LMRS	-0,810	0,842	1														
LMRT	-0,766	0,707	0,918	1													
LR	0,607	-0,270	-0,572	-0,541	1												
NF	-0,388	0,797	0,763	0,764	-0,281	1											
DMR	-0,451	0,679	0,496	0,315	-0,290	0,271	1										
NMf	-0,670	0,691	0,662	0,522	-0,511	0,423	0,798	1									
LMRF	0,780	-0,266	-0,629	-0,677	0,451	-0,269	-0,048	-0,283	1								
NI	0,532	0,220	-0,123	-0,143	0,417	0,377	-0,176	-0,195	0,523	1							
NRP	0,803	-0,291	-0,579	-0,594	0,575	-0,135	-0,410	-0,572	0,673	0,628	1						
NRS	0,826	-0,119	-0,494	-0,461	0,603	0,014	-0,314	-0,455	0,701	0,872	0,774	1					
NRT	0,410	0,329	-0,007	-0,004	0,303	0,462	-0,104	-0,080	0,373	0,946	0,473	0,798	1				
NML	-0,130	0,052	0,090	0,095	0,014	0,269	-0,160	-0,031	-0,136	0,025	0,171	-0,172	0,048	1			
NLCAP	-0,179	0,262	0,282	0,325	-0,357	0,254	0,153	0,161	-0,267	0,006	-0,058	-0,016	0,075	-0,020	1		
LPR	-0,529	0,576	0,492	0,378	-0,385	0,289	0,762	0,787	-0,142	-0,224	-0,478	-0,387	-0,153	-0,114	0,247	1	
LLR	-0,184	0,868	0,597	0,465	-0,051	0,722	0,541	0,485	0,088	0,495	0,058	0,248	0,526	-0,094	0,194	0,396	1

In fact, the morphological study of [27] showed variations among the 33 accessions of *Ricinus communis* L. from Andaman and Nicobar Islands for all the 18 traits studied. This work reveals also that plant height exhibited high significant positive correlations with the number of nodes on the main stem. In addition, the cluster analysis based on morphological traits grouped the 33 accessions of *Ricinus communis* L. into two major clusters [27].

Furthermore, the study of [28] was found a significant amount of genetic variability for all the twenty morphological parameters studied among safflower germplasm. In addition, this work reveals that seed yield plant had high significant and positive correlation with branches plant, capitulum plant, seeds capitulum and 100 seed weight. Furthermore, the hierarchical cluster analysis based on agro-morphological parameters divided the 121 accessions of safflower into 5 main clusters [28].

The morphological study of [29] in rice varieties showed high phenotypic variability ($P < 0.0001$) for the characters: leaf length and leaf width, primary branching, maturity and grain thickness. In addition, this work revealed a positive and strong correlation (0.77) between the height at maturity and leaf length. The cluster analysis of this morphological study based on Euclidien distances between the 98 genotypes of Rice has allowed identifying three major clusters.

4. Conclusion

The phenological study shows that the 3 species studied have distinct phenologies. The longest phenological cycle is observed for *Tanacetum parthenium.* The variance analysis showed significant differences to highly significant for the majority of the traits studied. Furthermore, this study allowed us to validate the morphological and phenological approach as tools for selection of suitable genotypes. This genetic diversity will be more evidenced using molecular markers. Although, the morphological descriptors of *Anacyclus clavatus*, *Chamaemelum fuscatum* and *Tanacetum parthenium* must be completed by a molecular analysis using RAPD, SSR or AFLP to understand the genetic organization of these species in Tunisia.

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