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Growth and Molecular Expression of Okra Seeds Interacted with Fourteen Mango Cultivars in Mixed Cropping System

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Abstract

The present study was accomplished to study the effect of fourteen cultivars (Keitt, Ewais, White Succari, Tommy Atkins, Fajri Klan, Zebda, Alphonso, Sedeek, Naomi, Mesk, Baladi Dabsha, Baladi Arnaba, Cobania and Totapuri) of *Mangifera indica* L. leaves aqueous extracts (MILAE) on germination and some growth parameters as well as seedling protein profile of *Hibiscus esculentus* L. (okra seeds) in mixed cropping system. The study was extented to characterize and discriminate among the aforementioned cultivars. The allelopathic potential of fourteen cultivars of *M. indica* leaves aqueous extracts (MILAE) on germination efficiency and growth parameters of *H. esculantus* L. seeds (as a recipient bioassay material) was completely studied. Hypocotyl length (HL) was more sensitive than radicle length (RL) for all studied cultivars. At the all concentrations level, Mesk cultivar exerted the highest allelopathic effect, while Totapuri cultivar showed the lowest one on the germination percentage (GP), inhibition percentage (IP), the time to get 50% germination (T_{50}), mean germination time (MGT), germination energy (GE), seed germination index (SGI), emergence percentage (EP), mean emergence time (MET), seedling emergence index (EI), seedling vigour index (SVI), hypocotyl (HL) and radicle (RL) lengths, seedling fresh (SFW) and dry (SDW) weights.

Seedling protein electrophoresis data revealed that Tommy Atkins cultivar attained the minimum values for both the number of bands and the percentage of polymorphism, 6 bands and 27%, respectively. On the other hand, the maximum values were achieved from both Naomi and Totapuri cultivars, 11 bands and 51%. Reversibly, the genomic template stability (GTS %) oscillated from 32% in Cobania cultivar to 64% in specimen Tommy Atkins cultivar. The resulted dendrograms by using allelopathic and molecular data as well as seedling protein electophoresis ascertains three aggregations. The first assembly includes Sedeek, Naomi and Mesk cultivars. The second gathers Keitt and Ewais cultivars. While, the third clusters Fajri Klan, Zebda and Alphonso cultivars.

Keywords: allelochemicals, *Hibiscus esculentus*, *Mangifera indica*, polycropping practices, seedling protein electrophoresis

1. Introduction

Nowadays, there is a large move from monocropping to polycropping practices, in almost of the agricultural ecosystems along the world. One should be aware by the chemical interfering between the mixed crops in order to avoid undesirable potential effects of one crop on the other. El-Kenany et al. (2014) assessed the probable effects of *Nigella sativa* L. seeds on the germination and growth alongside nutrient, metabolite and pigment content of *Lupinus termis* L. in mixed cropping system of the two crops. Multiple cropping is common practice in subtropical and tropical regions (Young et al., 1989; El-Kenany et al., 2014). Because there is a large move from mono-cropping to multiple cropping practices, one should be aware by the chemical interfering between the mixed crops in order to avoid undesirable potential effects of some crop on the others. A little is known about the allelopathic interaction of intercropped plants in mixed farming systems.

Mango (*Mangifera indica* L.), member of the cashew family (Anacardiaceae) is one of the most important and widely cultivated fruits of the tropical world. Mango attains high intraspecific diversity with about 1000-1600 cultivars in worldwide, of which 350 cultivars are in commercial production and the rest are limited to mixed orchards or home gardens (Bally, 2006; Aiyelaagbe & Osamudiamen, 2009). Mango production is concentrated

in El-Sharkia, El-Ismailia, El-Giza, EL-Fayoum and El-Behera (Nubariya) governorates (Abourayya et al., 2012). The lack of systematic approach in naming of mango cultivars in the past has resulted in a great confusion in their nomenclature due to many synonyms and duplication of names in the absence of any rules governing nomenclature (Kumar et al., 2013; Mansour et al., 2014).

Allelopathy is a physiological phenomenon with ecological implications and considered as an applicable technique in ecology (Reigosa et al., 2006). It is direct and indirect plant interactions mediated by allelochemicals (Cheema et al., 2013). An organism produces one or more biochemical (allelochemicals) influence the growth, survival and reproduction of other organisms. It also involves chemical interactions at all levels of complexity, from microorganisms to higher plants (Rice, 1974). When plants are exposed to allelochemicals, their growth and development are highly affected (Putnam & Duke, 1978; Niakan et al., 2008). Allelochemicals significantly interfered with the protein expression of the recipient species. This interference took place either by induction or repression of the protein bands. The relatively high frequency of disappearance of bands may reveal that the survival of the individuals was greatly affected by allelochemicals (El-Khawas and Shehata, 2005; Cenkci et al., 2010; Sunar et al., 2013).

Allelopathic potential consider as a differential marker among cultivars, varities and growth forms (El-Darier et al., 2014). The differential allelopathic potential of cultivars was positively correlated with the contents of total phenolic acids (Labbafi et al., 2010; Bertholdsson, 2012). El-Darier et al. (2015) indicated that the phytochemical screening of fourteen mango cultivars (Keitt, Ewais, White Succari, Tommy Atkins, Fajri Kalan, Zebda, Alphonso, Sedeek, Naomi, Mesk, Baladi Dabsha, Baladi Arnaba, Cobania and Totapuri) was a successful tool to discriminate among them. In addation, the variation in phenolics, flavonoids and mangiferin were highly specific for these cultivars.

Seedling storage proteins are powerful tool for the detection of the genetic diversity and also considered as reliable technology because these are highly independent on environmental fluctuations (Javid et al., 2004; Iqbal et al., 2005; Netra & Prasad, 2007; Sadia et al., 2009). Seedling storage protein profiling can be employed for various purposes, such as varietal and cultivar identification (El-Khawas & Shehata, 2005), biosystematics analysis, determination of phylogenetic relationship among different species and generation of related information to complement evaluation (Sammour, 1991; Isemura et al., 2001; Ghafoor et al., 2002).

The main objective of the present study was to demonstrate and verify the interfere of allelochemicals liberated from young leaves of fourteen mango cultivars with germination efficiency and growth parameters as well as seedling protein profile of okra in mixed cropping system. The study was extented to characterize and discriminate among the aforementioned cultivars.

2. Materials and Methods

2.1 Collection of Specimens

Leaves of fourteen cultivars of *M. indica* L. were collected from El-Sharkia governorate (about 235 Km east of Alexandria governorate). The cultivars were collected from two sites; El-salihiyyah Al-jadidah (50 Km from Al-Zakazik city) and El-sanagra village, Abu Hammad (15 Km from Al-Zakazik city). Nine cultivars were obtained from the first site, while five cultivars were gathered from the later site.

2.2 Preparation of M. indica Leaves Aqueous Extract (MILAE)

The collected leaves of the studied fourteen mango cultivars were washed and dried in an electric oven at 45 °C then ground to a fine powder. One-hundred ml of sterile deionized water were added to 1, 2, 4 and 8 g and the mixture was shaken and left for 48 h at refrigerator to 1, 2, 4 and 8% beside the control (distilled water). The procedures were performed according to El-Rokiek et al. (2010) and Algandaby et al. (2014).

2.3 Germination Bioassay

Petri-dish experiment was applied to investigate the potential allelopathic effects of *M. indica* leaves aqueous extract (MILAE) of fourteen different cultivars on germination percentage (GP), emergence percentage (EP), mean emergence time (MET) and seedling vigour index (SVI), hypocotyl (HL) and radicle (RL) lengths, seedling fresh (SFW) and dry (SDW) weights. Also the experiment will be extended to assess the probable allelopathic interference between okra and different mango cultivars in mixed culture practices.

To accomplish this experiment, 20 seeds of *H. esculentus* L. (okra) (recipient species) were arranged in 9-cm diameter Petri-dishes on two discs of whatman No. 1 filter paper under normal laboratory conditions. Ten ml of MILAE (1, 2, 4 and 8%) or distilled water as control were added daily to three replicates in a randomized complete block design. Half of the extract was used to moisten the bottom filter paper receiving the seeds while

the remaining half was applied after covering seeds with filter paper and Petri dishes were covered with lid. Petri-dishes were placed at room temperature, with day temperature ranging 25-30 °C and night temperature ranging 20-25 °C. The lid of Petri dish and the upper filter paper were removed just at initiation of seed germination and seedlings emergence. Seeds were considered to be germinated when their radicle was nearly 2 mm, while seedlings were assessed as emerged when the cotyledons had unfolded above the surface and hypocotyl length was nearly 2 mm. this procedure was described by Abdul Khaliq et al. (2012).

Before sowing, the seeds were immersed in 2% CHLOREX for 2 minutes then rinsed four times with distilled water. Finally, the seeds were soaked in aerated distilled water for 24 hours. GP, EP, HL and RL were recorded daily for successive sixteen days according to AOSA (1990). After sixteen days, the homogenous seedling were carefully collected from each treatment, washed with tap water and then by distilled water, gently blotted with filter paper. Fresh weight of seedlings was determined and then seedlings were dried at 65 ºC till constant weight to determine the dry weight.

2.4 Calculations and Data Analyses

(1) Mean Emergence Time (MET)

Mean emergence time (MET) was calculated according to the equation of Ellis and Roberts (1981).

$$\text{MET} = \Sigma \text{ Number of emerged seedlings/Day of counting} \quad (1)$$

(2) Seedling Vigour Index (SVI)

Seedling vigour index (SVI) was calculated according to the equation of Islam et al. (2009) and Elouaer and Hannachi (2012).

$$\text{SVI} = [\text{Seedling length (cm)} \times \text{Germination percentage}]/100 \quad (2)$$

2.5 Data Analysis and Computer Programs

Data were subjected to standard analysis of variance (ANOVA) using COSTAT 2.00 statistical analysis software manufactured by Cohort software company (Zar, 1984). In addition, data were subjected to numerical analysis using PAST program for mixed data set (Hammer et al., 2001). The agglomerative cluster analysis was conducted by using "Euclidean" coefficient (Hammer et al., 2005) through the Unweighed Pair Group Method using Arithmetic averages (average linkage, UPGMA) method of sorting to estimate the relationships among the fourteen cultivars.

Euclidean Distance Coefficient:

$$\text{Euclidean}_{jk} = \sum_{i=1}^{S} \left(X_{ij} - X_{ik}\right)^2 \quad (3)$$

Where,

S = number of species being compared, X_{ij} = frequency of species j in sample i (i = sample number 1, 2, etc), X_{ik} = frequency of species k in sample i (i = sample number 1, 2, etc).

2.6 Growth Experiment

Growth experiment was performed to test the allelopathic effect of high concentration level (8%) of *M. indica* leaves aqueous extract (MILAE) of the studied cultivars with sandy clay soil on seedling protein electrophoresis as molecular marker.

The soil samples were finally sterilized at (90 ºC for 48 h) to remove any microorganisms and weed seeds. Twenty seeds of *H. esculentus* were sown in plastic pots (16 cm in diameter) with about 1500 g of sandy clay soil. Ten ml of MILAE (8%) and distilled water as control were added daily to three replicates in a randomized complete block design. Before sowing, the seeds were immersed in 2% CHLOREX for 2 minutes then rinsed four times with distilled water. Finally, the seeds were soaked in aerated distilled water for 24 hours. The experiment was performed under normal laboratory conditions (20±2 ºC temperature, 75±2% relative humidity, and 14/10 h/dark photoperiod).

After twenty one days, the homogenous seedling were carefully collected then washed with tap water to remove the adhering soil particles, and then, by distilled water, gently blotted with filter paper. Shoot system prepared and concealed with foil paper then put in ice and used for seedling protein electrophoresis experiment.

2.7 Seedling Protein Electrophoresis

For assessing the allelopathic effect of the fourteen cultivars of *M. indica* leaves aqueous extract (MILAE) at 8% concentration, on the protein content of *H. esculentus* seedling, sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) was carried out according to Laemmli (1970).

2.8 Data Analysis and Computer Programs

The bands produced by each sample were counted and the percentage of polymorphism was determined according to the following equation:

$$\text{The percentage of polymorphism} = [(\Sigma \text{ Bands for each sample}/\Sigma \text{ Bands for all samples}] \times 100 \quad (4)$$

Genomic template stability (GTS %) was calculated as the following:

$$GTS\ \% = (1 - a/n) \times 100 \quad (5)$$

Where, a: average number of polymorphic bands detected in each treated sample, (polymorphism include appearance of new bands and disappearance normal bands), n: total number of bands (Cimino, 2006).

The different molecular weights were determined by means of UVP Doc-It®LS image Analysis Software, and employed in the analysis by using PAST program (Hammer et al., 2001) to compute the genetic distances and generate the dendrogram. The agglomerative cluster analysis was conducted by using "Chord" coefficient (Hammer et al., 2005) through the Unweighed Pair Group Method using Arithmetic averages (average linkage, UPGMA) method of sorting.

Chord Coefficient:

$$Chord_{jk} = \sqrt{2\text{-}2\ \frac{\sum_{i=1}^{S} (X_{ij}\, X_{ik})}{\sqrt{\sum_{i=1}^{S} X_{ij}^{2}\ \sum_{i=1}^{S} X_{ik}^{2}}}} \quad (5)$$

Where,

S = number of species being compared, X_{ij} = frequency of species j in sample i (i = sample number 1, 2, etc), X_{ik} = frequency of species k in sample i (i = sample number 1, 2, etc).

3. Results

3.1 Germination Efficiency

Data concerning the germination percentage (GP), emergence percentage (EP), mean emergence time (MET) and seedling vigour index (SVI) of *H. esculentus* L. are illustrated Table 1. Petri-dish experiment demonstrated that the GP and EP of *H. esculentus* was significantly ($p \leq 0.05$) affected upon applying different concentration levels of fourteen cultivars of *M. indica* L. leaves aqueous extract (MILAE). Commonly, GP and EP decreased with the increase in MILAE concentration. At the end of the experiment (after sixteen days) GP and EP attended a value of about 100 % at control level for all the fourteen studied cultivars. However, at the consequent concentrations (1, 2, 4 and 8%) the values were significantly decreased and prominent in EP relative to GP.

On contrarily to the last mentioned germination parameters (GP and EP), the value of the mean emergence time (MET), increased markedly as MILAE concentration increased, MET are illustrated and statistically represented in**.** This increase was statistically significant at ($p \leq 0.05$) as evaluated by ANOVA test. At control level, the value of MET was 1 respectively for all studied cultivars. In MET the values ranged from a minimum in Totapuri cultivar and a maximum in Mesk cultivar. At 2% MILAE concentration, values of MET ranged from a minimum in Totapuri cultivar and a maximum in Mesk cultivar. At 4% MILAE concentration, values of MET ranged from a minimum in Totapuri cultivar and a maximum in Mesk cultivar. In MET a notable increase was attained along the higher MILAE concentration (8%), in MET the values were increased in Mesk cultivar to a maximum values of about 11.1.

The values of seedling vigour (SVI) index, decreased distinctly as MILAE concentration increased. SVI was illustrated and statistically represented in Table 1. This reduction was statistically significant at ($p \leq 0.05$) as evaluated by ANOVA test. At control level, while SVI attain a value of about 20 for all studied fourteen cultivars. At (1, 2, 4 and 8%) MILAE concentration SVI conquered a value ranged from a minimum in Mesk cultivar to a maximum in Totapuri cultivar.

Table 1. Variation in germination percentage (GP), emergence percentage (EP), mean emergence time (MET) and seedling vigour index (SVI) of *Hibiscus esculentus* L. seeds (sixteen days after sowing) as affected by different concentration levels (%) of fourteen cultivars of *Mangifera indica* leaves aqueous extract (MILAE). Different letters within each column for each cultivar indicate a significant difference at $p < 0.05$ according to one way ANOVA test

Germination parameter	Treatment (%)	Keitt	Ewais	White Succari	Tommy Atkins	Fajri Klan	Zebda	Alphonso	Sedeek	Naomi	Mesk	Baladi Dabsha	Baladi Arnaba	Cobania	Totapuri
GP	**C**	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}
	1	96^{b}	94^{b}	91^{b}	98^{b}	88^{b}	97^{b}	88^{b}	86^{b}	83^{b}	80^{b}	95^{b}	96^{b}	97^{b}	99^{b}
	2	90^{c}	88^{c}	89^{c}	93^{c}	84^{c}	90^{c}	80^{c}	75^{c}	77^{c}	73^{c}	91^{b}	92^{c}	92^{c}	95^{c}
	4	74^{d}	84^{d}	79^{c}	86^{d}	76^{d}	85^{d}	70^{d}	69^{d}	67^{d}	60^{d}	88^{c}	85^{d}	88^{d}	91^{d}
	8	62^{e}	76^{e}	73^{d}	77^{d}	69^{e}	77^{d}	65^{e}	60^{e}	63^{e}	50^{e}	80^{d}	78^{e}	83^{d}	86^{e}
EP	**C**	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}
	1	65^{b}	70^{b}	76^{b}	80^{b}	69^{b}	79^{b}	71^{b}	55^{b}	57^{b}	45^{b}	68^{b}	80^{b}	82^{b}	85^{b}
	2	55^{c}	63^{c}	65^{c}	69^{c}	57^{c}	66^{c}	56^{c}	40^{c}	39^{c}	36^{c}	54^{c}	66^{c}	70^{c}	74^{c}
	4	38^{d}	49^{d}	50^{d}	55^{d}	43^{d}	45^{d}	31^{d}	33^{d}	30^{d}	25^{d}	39^{d}	52^{d}	60^{d}	63^{d}
	8	25^{e}	32^{e}	34^{e}	39^{e}	28^{e}	30^{e}	23^{e}	20^{e}	18^{e}	15^{e}	22^{e}	37^{e}	40^{e}	45^{e}
MET	**C**	1.0^{a}	1.0^{a}	1.0^{a}	1.0^{a}	1.0^{a}	1.0^{a}	1.0^{a}	1.0^{a}	1.0^{a}	1.0^{a}	1.0^{a}	1.0^{a}	1.0^{a}	1.0^{a}
	1	1.6^{b}	1.4^{b}	1.2^{b}	1.2^{b}	1.4^{b}	1.2^{b}	1.4^{b}	1.7^{b}	1.6^{b}	2.1^{b}	1.4^{b}	1.2^{b}	1.1^{b}	1.1^{b}
	2	2.0	1.6^{c}	1.7^{c}	1.5^{c}	1.8^{c}	1.7^{c}	2.2^{c}	2.9^{c}	3.0^{c}	3.2^{c}	2.1^{c}	1.4^{c}	1.3^{c}	1.2^{c}
	4	3.4^{d}	2.3^{d}	2.2^{d}	2.0^{d}	2.3^{d}	2.6^{d}	4.2^{d}	3.7^{d}	5.0^{d}	6.2^{d}	3.1^{d}	2.4^{d}	2.1^{d}	1.9^{d}
	8	5.5^{e}	3.5^{e}	3.8^{e}	3.4^{e}	5.0^{e}	4.5^{e}	6.6^{e}	7.7^{e}	9.0^{e}	9.1^{e}	6.1^{e}	3.7^{e}	2.9^{e}	2.6^{e}
SVI	**C**	20.5^{a}	20.5^{a}	20.5^{a}	20.5^{a}	20.5^{a}	20.5^{a}	20.5^{a}	20.5^{a}	20.5^{a}	20.5^{a}	20.5^{a}	20.5^{a}	20.5^{a}	20.5^{a}
	1	10.1^{b}	11.9^{b}	9.9^{b}	13.3^{b}	8.0^{b}	10.7^{b}	7.5^{b}	6.6^{b}	5.9^{b}	5.4^{b}	8.7^{b}	10.1^{b}	11.6^{b}	13.9^{b}
	2	8.6^{c}	10.2^{c}	9.1^{c}	10.4^{c}	6.5^{c}	8.6^{c}	6.5^{c}	5.2^{c}	4.6^{c}	4.4^{c}	7.7^{c}	8.8^{c}	8.2^{c}	11.9^{c}
	4	6.0^{d}	8.4^{d}	6.7^{d}	8.6^{d}	5.0^{d}	5.4^{d}	5.0^{d}	4.1^{d}	3.5^{d}	31.8^{d}	6.3^{d}	6.6^{d}	6.8^{d}	8.8^{d}
	8	3.7^{e}	6.8^{e}	4.2^{e}	4.9^{e}	3.5^{e}	3.6^{e}	2.2^{e}	3.1^{e}	2.9^{e}	2.1^{e}	4.9^{e}	4.6^{e}	5.7^{e}	6.5^{e}

3.2 Hypocotyl (HL) and Radicle (RL) Lengths

The allelopathic effect of MILAE concentration on hypocotyl (HL) and radicle lengths of okra are illustrated and represented as exponential regression in Figure 1. MILAE extract concentration levels have statistically reduced both hypocotyl (HL) and radicle lengths. The applied concentrations are significant at $p \leq 0.05$. Hypocotyl length of *H. esculentus* was more sensitive than radicle length as affected by different concentration levels of all studied cultivars of *M. indica* leaves aqueous extracts. Apparently, HL and RL decreased with the increase of MILAE concentration in all fourteen cultivars (negative exponential relationship) where $R^2 = 0.9$. After sixteen days from sowing, HL and RL attended a gradual reduction at (control level, 1%, 2%, 4% and 8%) for the fourteen studied cultivars. The values were significantly decreased and prominent in Mesk cultivar relative to Totapuri cultivar.

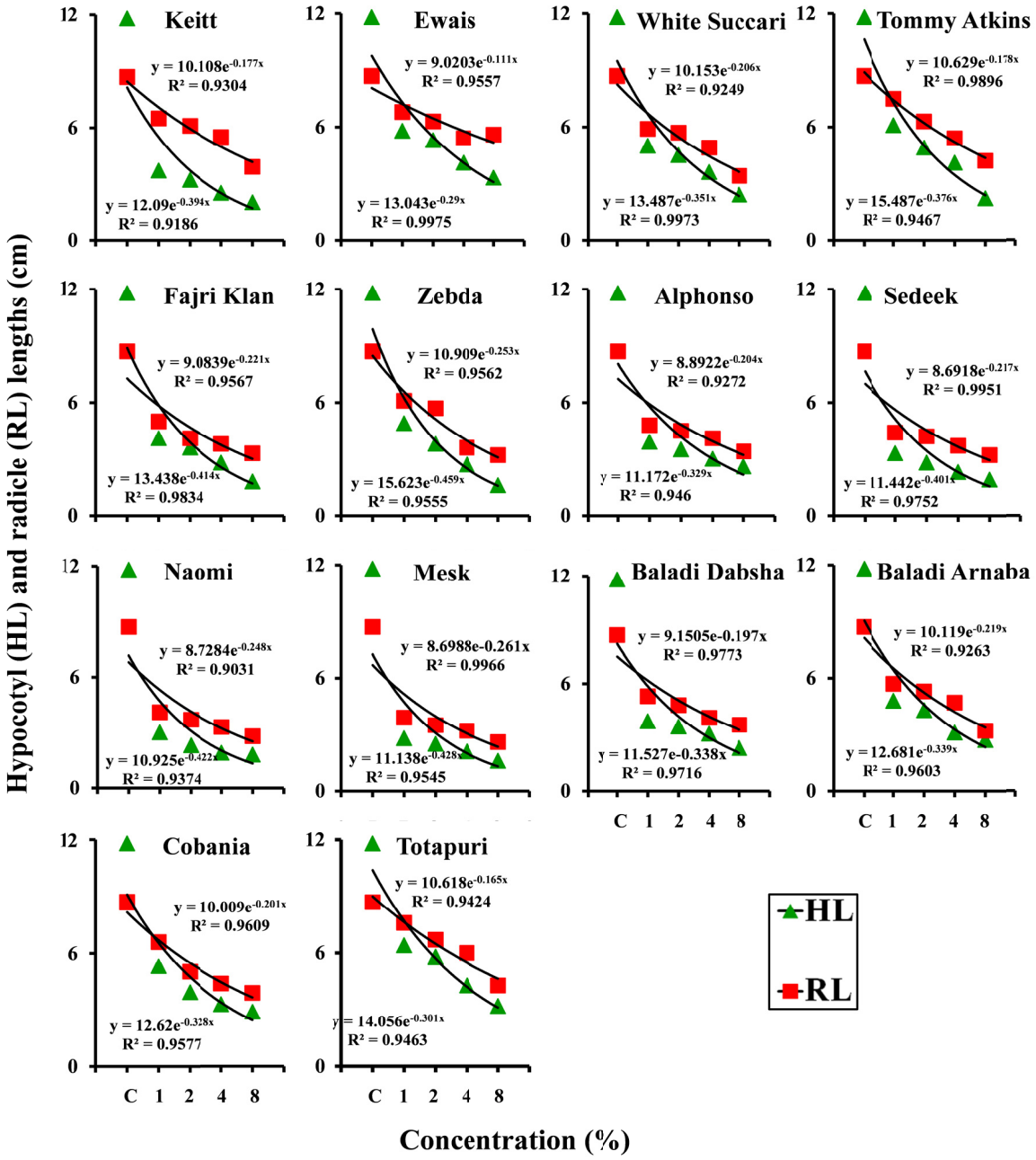

Figure 1. Exponential regression of hypocotyl (HL) and radicle (RL) lengths of *Hibiscus esculentus* seeds (sixteen days after sowing) as affected by different concentration levels (%) of fourteen cultivars of *Mangifera indica* leaves aqueous extracts

3.3 Fresh (SFW) and Dry (SDW) Weights

The allelopathic effect of MILAE concentration on seedling fresh (SFW) and dry (SDW) weights of *H. esculentus* L. are illustrated and represented as exponential regression in Figure 2. MILAE extract concentration levels have statistically reduced SFW and SDW. The applied concentrations are significant at $p \leq 0.05$. Usually, SFW and SDW decreased with the increase of MILAE concentration for all studied cultivars (negative exponential relationship) where $R^2 = 0.9$. SFW and SDW attended a gradual reduction at (control level, 1%, 2%, 4% and 8%) for the fourteen studied cultivars. The values were significantly decreased and prominent in Mesk cultivar relative to Totapuri cultivar.

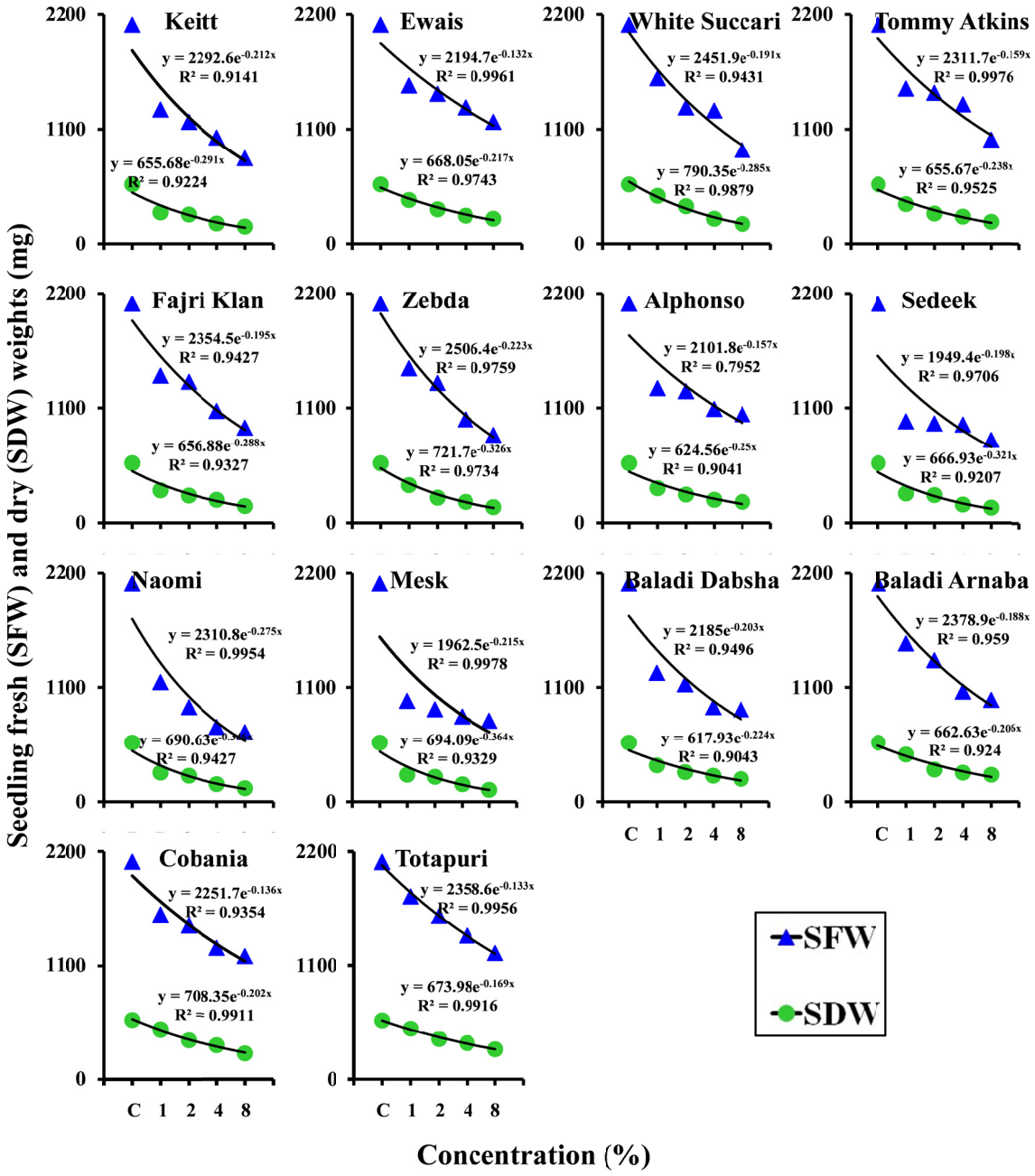

Figure 2. Exponential regression of seedling fresh (SFW) and dry (SDW) weights of of *Hibiscus esculentus* seeds (sixteen days after sowing) as affected by different concentration levels (%) of fourteen cultivars of *Mangifera indica* leaves aqueous extracts

The results obtained from the fourteen studied cultivars of mango are subjected to numerical analysis to discriminate among them. The use of the allelopathic characters emergence percentage (EP), mean emergence time (MET), seedling vigour index (SVI), hypocotyl (HL) and radicle (RL) lengths, fresh (SFW) and dry (SDW) weights provide a reliable dendrogram rather than using each character separately or collectively (germination and reduction characters). This is attributed to avoiding the logically correlated characters to be used in the numerical analysis. The dendrogram based on allelopathic characters discriminates studied cultivars into three aggregations. The first assembly includes Sedeek, Naomi and Mesk cultivars. The second gathers Keitt and Ewais cultivars. While, the third clusters Fajri Klan, Zebda and Alphonso cultivars (Figure 3).

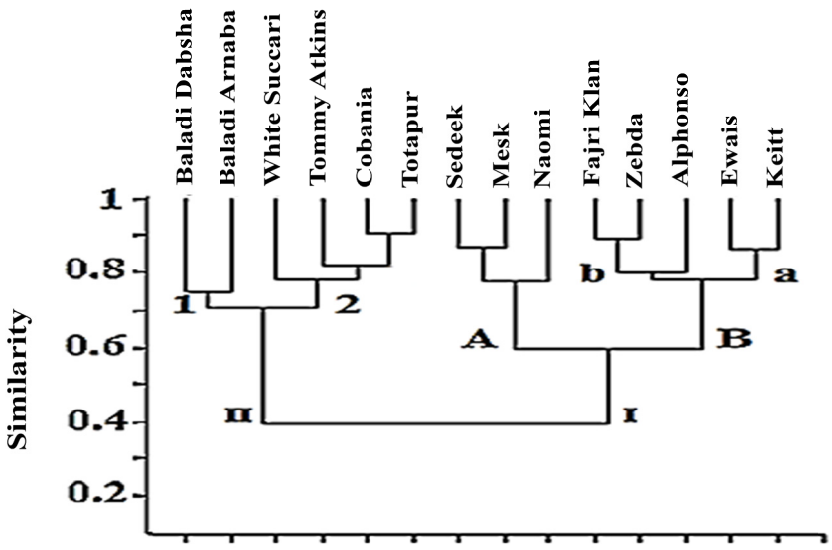

Figure 3. Dendrogram resulting from UPGMA method of sorting using the allelopathic effect of fourteen cultivars of *Mangifera indica* L. leaves aqueous extract under different concentration levels (Control, 1, 2, 4 and 8%) on *Hibiscus esculentus* seeds

3.4 Seedling Protein Electrophoresis

The electrograms of the fourteen *H. esculentus* specimens, which were subjected to the allelopathic effects of the fourteen mango cultivars, are illustrated in Figure 4. However, the constructed dendrogram elucidated the relationships among them was declared in Figure 5. A total of 22 bands are produced without both common or specific bands. The specimen 4 (subjected to Tommy Atkins cultivar) attained the minimum values for both the number of bands and the percentage of polymorphism, 6 bands and 27%, respectively. On the other hand, the maximum values were achieved from both specimens "9" and "14" (subjected to Naomi and Totapuri cultivars), 11 bands and 51% (Table 2). The studied specimens manifested the appearance and the disappearance of bands, as well as the alteration bands intensities. Both the appearance and disappearance of bands fluctuated from 8 to 15 bands in specimens "4" (subjected to Tommy Atkins cultivar) and "9" (subjected to Naomi cultivar), respectively. Reversibly, the genomic template stability (GTS %) oscillated from 32% in specimen "11" (subjected to Cobania cultivar) to 64% in specimen "4" (subjected to Tommy Atkins cultivar) (Table 3).

The constructed dendrogram based on seedling proteins divaricated at 0.15 similarity level, into two major groups "I" and "II". The major group "I" includes 5 specimens affective by cultivars; Keitt, Ewais, Fajri Klan, Zebda and Alphonso. Similarly, in the major group "II", the specimens subjected to Sedeek, Naomi and Mesk cultivars are clustered together as in the dendrogram based on allelopathic characters (Figure 5).

Table 2. Details of the seedling protein patterns for the study species *Hibiscus esculentus* L. affected by 8% concentration of fourteen cultivars of *Mangifera indica* L. leaves aqueous extract

Characters \ Studied cultivars	Control	Keitt	Ewais	White Succari	Tommy Atkins	Fajri Klan	Zebda	Alphonso	Sedeek	Naomi	Mesk	Baladi Dabsha	Baladi Arnaba	Cobania	Totapuri
Total number of bands for each cultivar	9	8	9	7	6	10	8	8	8	11	8	9	9	10	11
Percentage of polymorphism (%)	41	37	41	32	27	46	37	37	37	51	37	41	41	46	51

Table 3. Changes of both total and polymorphic bands in *Hibiscus esculentus* L. affected by 8% concentration of fourteen cultivars of *Mangifera indica* leaves aqueous extract; a, indicates appearance of new bands; b, indicates disappearance of normal bands; c, decrease in band intensities; d, increase in band intensities; a + b, denotes polymorphic bands; a + b + c + d, varied band and GTS%, Genomic template stability

Studied cultivars	Bands						GTS%
	a	b	c	d	a+b	a+b+c+d	
Control	0	0	0	0	0	0	100%
Keitt	5	6	2	1	11	14	50%
Ewais	6	6	2	1	12	15	46%
White Succari	6	7	2	0	13	15	41%
Tommy Atkins	2	6	2	1	8	11	64%
Fajri Klan	7	6	3	0	13	16	41%
Zebda	4	6	3	0	10	13	55%
Alphonso	5	6	3	0	11	14	50%
Sedeek	5	6	2	1	11	14	50%
Naomi	8	6	3	1	14	18	36%
Mesk	5	6	3	0	11	14	50%
Baladi Dabsha	7	7	2	0	14	16	36%
Baladi Arnaba	4	5	2	1	9	12	59%
Cobania	8	7	2	0	15	17	32%
Totapuri	7	5	3	1	12	16	45%

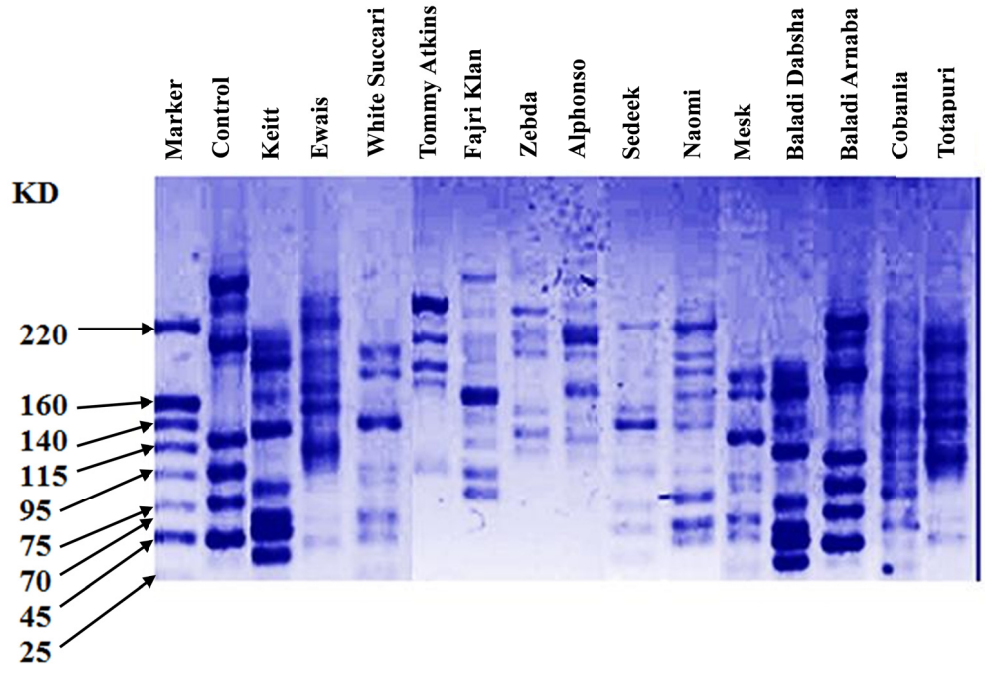

Figure 4. Seedling protein electrophoresis obtained from the leaves of *Hibiscus esculentus* L. affected by 8% concentration level of the fourteen cultivars of *Mangifera indica* L. leaves aqueous extract

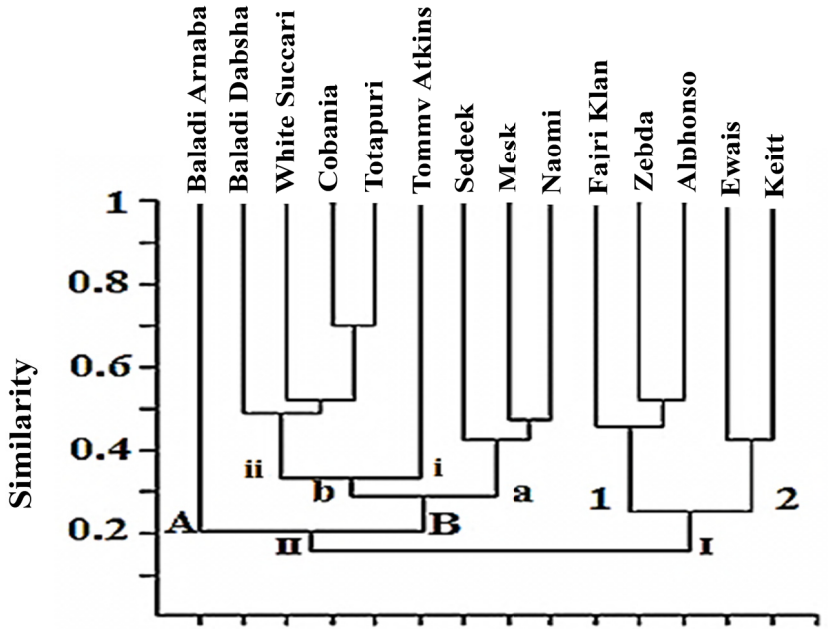

Figure 5. Dendrogram resulting from UPGMA method of sorting using the seedling protein electrophoresis obtained from the leaves of *Hibiscus esculentus* affected by 8% concentration level of the fourteen cultivars of *Mangifera indica* L. leaves aqueous extract

4. Discussion

In the present study, the allelopathic potential of fourteen cultivars of *M. indica* leaves aqueous extracts (MILAE) under different concentrations levels (1, 2, 4 and 8% compare to control) on germination (GP) and (EP) of *H. esculentus* L. was confirmed (as a recipient species). Data suggested that the GP and EP of *H. esculentus* seeds was differentially affected by MILAE and the reduction was concentration dependent, these assessments are in a harmony with Sazada et al. (2009), Sahoo et al. (2010), Aamir and Ibrahim (2013), Khan et al. (2013), and Ma et al. (2014). Reduction in GP and EP, also may be due to different allelochemicals present in MILAE such as mangiferin, saponin, steroids, tannin, flavonoid, reducing sugars, cardiac glycosides and different phenolics (Aiyelaagbe & Osamudiamen, 2009; Luo et al., 2012; El-Darier et al., 2015). These compounds have been reported to be active allelopathic chemicals effect on GP and EP (El-Rokiek et al., 2011; Algandaby et al., 2014), where phenolic compounds inhibit the activity of GA or inhibit the synthesis of GA which regulate de novo amylase production during seed germination (Chandler et al., 1984; Einhellig, 1996). Inhibition percentage (IP) of GP and EP in the *H. esculentus* increased gradually with the increase of MILAE concentration levels. These results agree with Abou-Zeid and El-Darier (2014) and El-Darier et al. (2014). Alam and Islam (2002) and Amoo et al. (2008) suggested that the inhibition percentage (IP) of crop plants may be due to the disturbance in many physiological processes in receptor plants as well as activities of peroxidase, alpha-amylase and acid phosphates, which resulting in a reduction of plant growth and development with subsequent yield reduction.

A gradual increase in the mean emergence time (MET) of the recipient species as a response to the regular applying of higher MILAE concentration levels was attained. At the full-strength concentration (8%) Mesk cultivar exerts the highest allelopathic effect on MET of *H. esculentus*. This result is in agreement with Abdul Khaliq et al. (2012). The highest MET increases when the aqueous extract of MILAE concentration increased which showed that increased MILAE concentration caused a decrease in germination velocity (Modhej et al., 2013; Noghondar & Azizi, 2013). Results of seed germination seedling vigour (SVI) index indicated that a gradual reduction of all germination indices in the recipient species as a response to the regular applying of higher MILAE concentration levels was attained. At the full-strength concentration (8%) Mesk cultivar exerts the highest allelopathic effect on the SVI of *H. esculentus* seeds. SVI assessments are in a harmony with Tanveer et al. (2010) and Ali et al. (2012), they found a gradual reduction in SVI *Triticum aestivum*, *Cicer arietinum*, and *Lens culinaris* relative to mungbean as a response to the higher concentration levels of *Euphorbia helioscopia* and *Rhynchosia capitata* aqueous extract respectively.

The current study inferred that the hypocotyl length (HL) of *H. esculentus* was found more sensitive and responds more strongly to the increase in MILAE concentration than the radicle length (RL). These results are in concordance with El-Rokiek et al. (2010), Khan et al. (2013), Algandaby et al. (2014), and El-Darier et al. (2014). On contrary, Turk and Tawaha (2002, 2003), Ashrafi et al. (2008), and El-Darier and Zein El-Dien (2011) reported that water extracts of allelopathic plants had more pronounced effects on radicle than on hypocotyl growth. The reduction in both radicle length and hypocotyl length may be due to phytotoxic activity of phytochemicals present in aqueous extracts of *M. indica* leaves like mangiferin, saponin, steroids, tannin, flavonoid, reducing sugars and cardiac glycosides (Jutiviboonsuk & Sardsaengjun, 2010; Bhuvaneswari, 2013). It also contains phenolics like polyphenols, ferulic, cumaric acid, benzoic, vanelic, chlorogenic, caffeic, gallic, hydroxybenzoic, phenylpropanoids, mangiferin, mangin and cinnamon, These phenolic compounds have interfered with the phosphorylation pathway or inhibiting the activation of Mg^{2+} and ATPase activity or might be due to decreased synthesis of total carbohydrates, proteins and nucleic acids (DNA and RNA) or interference in cell division, mineral uptake and biosynthetic processes (El-Rokiek et al., 2010; Silva et al., 2012; El-Darier et al., 2015).

Results of both seedling fresh (SFW) and dry (SDW) weights indicated that a gradual reduction of (SFW) and dry (SDW) weights in the recipient species as a response to the regular applying of higher MILAE concentration levels was attained. At the full-strength concentration (8%) Mesk cultivar exerts the highest allelopathic effect on the (SFW) and dry (SDW) weights of *H. esculentus* seeds; these results are in concordance with those reported by Barkatullah et al. (2010) and Pirzad et al. (2010).

4.1 Seedling Protein Electrophoresis

In the present study the potential of seedling storage protein to assess the genetic diversity among the fourteen *H. esculentus* seedling affected by 8% *M. indica* leaves aqueous extract (MILAE) of the fourteen cultivars. This electrophoretic technique is commonly used onto taxa that are phenotypically closely related (Galani et al., 2011), specifies as a beneficial method for discrimination of several genotypes (Agafonov et al., 2001) and characterization among cultivars (Rao et al., 2013; Dar et al., 2014). It is obvious through the estimation of the genomic template stability percentage, which are relatively low in comparison with the control. This is in concordance with El-Khawas and Shehata (2005) and Hegazy et al. (2007) that the seedling proteins decrease with the increasement in the concentration of allelochemicals. This reduction may be due to the presence of phenolic compounds, which reduce the incorporation of phosphorus into DNA and RNA. Meanwhile, Baziramakenga et al. (1997) and Padhy et al. (2000) concluded that phenolic acids reduced the incorporation of certain amino acid into proteins and thus reduce the rate of protein synthesis. Allelochemicals significantly interfered with the protein expression of the recipient species (*H. esculentus*). These allelochemicals could play an important role in inhibiting enzymes involved in these two processes accordance with Baziramakenga et al. (1997) who pointed out that the methionine incorporation into proteins was reduced by allelochemicals. Furthermore, it is notable the intensity of protein bands decrease or increase depended on type of allelochemicals found in the fourteen mango cultivars, which affected on the recipient species (*H. esculentus*) (El-Khawas & Shehata, 2005; Sunar et al., 2013; Yumnamcha et al., 2014).

5. Conclusion

The present study was accomplished to study the effect of fourteen cultivars (Keitt, Ewais, White Succari, Tommy Atkins, Fajri Klan, Zebda, Alphonso, Sedeek, Naomi, Mesk, Baladi Dabsha, Baladi Arnaba, Cobania and Totapuri) of *Mangifera indica* L. leaves aqueous extracts (MILAE) on germination and some growth parameters as well as seedling protein profile of *Hibiscus esculentus* L. (okra seeds) in mixed cropping system. The study was extented to characterize and discriminate among the above-mentioned cultivars. This study recommends multiples cropping system by planting okra beside Totapuri mango cultivar depending on allelopathic, seedling protein electrophoresis data, that return to the low allelopathic effect of Totapuri cultivar due to absence of many phytochemicals such as alkaloids, flavonoids, saponins, steroids, tannins, phenolics, glycosides, triterpenoids, amino acids, carbohydrates and reducing sugar, also recorded low amount of quercetin, mangiferin and tannins. Okra seedling affected by Totapuri cultivar exerted high percentage of genome template stability (GTS %) of about 45%, while avoid planting okra seeds beside Mesk cultivar because this cultivar exert the highest allelopathic effect due to presence of mamy effective phytochemicals. Also, the resulted dendrogram depended on allelopathic data and the other dendogram subject to seedling protein elecrtrophoresis data ascertains three aggregations. The first assembly includes Sedeek, Naomi and Mesk cultivars. The second gathers Keitt and Ewais cultivars. While, the third clusters Fajri Klan, Zebda and Alphonso cultivars.

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