# Epidemiology and the Association of the *Fusarium* Species with the Mango Malformation Disease in Egypt

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Mango malformation disease (MMD) is an economically important disease of Mangifera indica Abstract: globally. This disease is caused by a complex of fungal pathogens, of which various *Fusarium* spp. dominate. This study was conducted to assess the epidemiology and its pathogenesis of mango malformation disease in Egypt. In three main Governorates of mango production, El Giza, Esamalîa and El-Bohera, disease incidence reached up to 80%. Maximum infection of traditional cultivars was observed in Hindi Sennara, Alfonso, Timour and Zebda. Exotic Tomy, Keet and Kent cultivars appeared to be moderate infection. Nine additional taxa have been isolated, i.e., F. subglutinans, F. oxysporum, F. sterilihyphosum, F. proliferatum, F. culmorum, F. nygamai, F. pseudonygamai, F. nelsonii and F. verticilioides from Egypt. Fusarium subglutinans proved to have the high frequency in all mango cultivars in tested area, while, F. oxysporum, F. sterilihyphosum, F. proliferatum frequently were less. To date, Koch's postulates have been applied with Fusarium for their pathogenic potential on mango cultivars seedlings under greenhouse conditions. Apparently, not all isolates of this Fusarium species are equally virulent on mango seedlings. Fusarium subglutinans proved to be the dominant fungus in all varieties. At the same time, F. oxysporum, F. sterilihyphosum, F. proliferatum, displayed also moderate virulence. Moreover, isolates colonized seedling root systems and became systemic, spreading to above-ground plant tissues include apical and lateral buds. Fusarium subglutinans proved to be the dominant fungus. Complex Strains of F. subglutinans, F. oxysporum, F. sterilihyphosum and F. proliferatum induced typical malformation symptoms on mango seedlings and trees in Egypt [Nature and Science. 2010;8(4):128-135]. (ISSN: 1545-0740).

Key words: Egypt, F. subglutinans, F.oxysporum, Fusarium sterilihyphosum and F. proliferatu, Mango Malformation, Mangifera indica.

#### 1. Introduction

Mango (Mangifera indica L.) is universally considered one of the most important fruit crop in tropical and subtropical areas of the world. Major producers include India, Pakistan, Brazil, Australia, South Africa, Egypt, and USA (Ploetz et al., 2002). Egypt produce 232,000 tone of mangos annually and export moderate amounts (1500 tones) to 20 countries in the near East and Europe . Mango suffers from several diseases at all stages of its life (Ploetz., 2003). Malformation is the most threatening disease causing colossal losses every year (Iqbal et al., 2006). Mango malformation disease (MMD), was first recorded in India in 1891. It is found elsewhere in Asia (Israel, Malaysia, and Pakistan), Africa (Egypt, South Africa, Sudan, Swaziland, and Uganda), and the Americas (Brazil, El-Salvador, Mexico, the United States, and Venezuela) (Marasas et al., 2006). The disease is endemic as a tree once infected never recovered. mango malformation can be classified into vegetative and floral malformation. (Zheng and Ploetz 2002). Three main symptoms of this phenomenon were recorded in Egypt i.e., malformed and stunted growth of seedlings in the nursery stage, vegetative growth malformation and inflorescence malformation in the bearing trees. The earliest hypothesis that mites caused the disorder did not last long as acaricides failed to control the problem (Yadav, 1999). Symptoms of the disease include loss of the apical dominance and swelling of vegetative buds, proliferation of leaves and flowers, phyllody and hypertrophy of panicle axes. The vegetative deformation may also affect immature trees and nursery stock, which can lead to the spread of infected plants. More important, however, is the affect of malformation on fruit set: fruit in affected panicles either do not set or abort. Primary and secondary axes on affected panicles are shortened, thickened and greatly branched (Kumar et al., 1993). The disease has been associated with physiologic disorders and hormonal imbalances (Singh et al., 1991 and Tapan et al., 2006) and attacks of an eriophyid mite, Aceria

(Eriophyes) mangifera (Doreste, 1984). However, Fusarium subglutinans [Gibberella fujikuroi var. subglutinans ] appears to have a significant role in malformation. Koch's postulates have only been completed for Fusarium subglutinans and F. oxysporum (Covarrubias, 1989) as the causal agents of malformation. Yet some controversy remains regarding species identification and the inoculation methods used . In 2002, a new species, F. mangiferae, was established based on nuclear and mitochondrial DNA sequences; it included strains of F. subglutinans from Egypt, Oman, Florida, Israel, Malaysia, and South Africa, some of which had been shown to cause MMD by artificial inoculation (Ploetz et al., 2002 and Kvas et al., 2008). Three or more additional taxa have been associated with MMD: F. sterilihyphosum from Brazil and South Africa, and Fusarium sp. nov. and F. proliferatum (teleomorph: Gibberella intermedia) from Malaysia (Marasas, et al., 2006 and Alvarado et al., 2006). Currently, the disease has spread where mangos are grown and causes the most severe damage in Egypt (Ploetz et al., 2002 and Haggag, Wafaa and Abd Wahab, 2009).

The present study had two objectives: (i) to determine the frequency of different fungi associated with malformed tissues and establish the cause of mango malformation in Egypt, and (ii) to evaluate pathogenic potential of selected *Fusarium* isolates in mango malformed diseased plants. Tests were conducted under controlled conditions on seedlings in a greenhouse.

## 2.Materials and Methods

## 2.1. Disease survey.

A disease survey in El-Behera, El-Giza and Esamali Governorates was performed on complete differentiation of healthy and malformed plants during the vegetative and flowering growth cycle (December to July) of 2008 season. Six traditional varieties (Hindi Sennara, Alfonso, Timour, Zebda, Awais and Dabsha) and three exotic verities (Keet, Kent and Tomy) were kept under the study. Each location contributed five panicles along with 6-8 cm shoot portion representing one of each variety. From each of the 5 districts, 10 samples of every cultivar were collected. Tissue pieces 5 mm long, were surface sterilized in 1% NaOCl solution for 2 minutes and placed onto Potato Dextrose agar (PDA) medium in 9 cm diameter Petri plates. The plates were kept in a incubator at 25°C under fluorescent illumination to give a 12 hour photoperiod to ensure maximum macroconidial production. After 6-7 days of incubation, the isolated fungi were identified on the basis of morphological characters (Summerell et al. 2003).

## Disease assessment.

Three branches at the four cardinal points were labeled per tree canopy. During each assessment, the total number of healthy and diseased shoots (vegetative and floral) were counted on each branch and averaged over the three branches per tree. The disease progress was determined as the accumulated proportion of diseased shoots per tree (*Yic*) corrected for host growth. At each time, (*i*), *Yic* was calculated as: *Yic* = *Yi/N*, in which, *Yi* is the accumulated number of diseased shoots at time *I* and *N* is the total number of weekly in the vegetative and floral stage (January to May) of mango growth .

## 2.2.Disease progress.

The experiment was conducted during 2008 growing cycles in a 6-year-old commercial orchard of the mango cultivars in the Noubaria station, Behera Govarnorate . The soil was -sandy, lightly compacted one . Trees averaged 3m height, with a mean trunk diameter of 0.5 m and a spacing of 5 m between tree rows. The trees had an average of 25 floral and vegetative deformations at the beginning of the experiment. A randomized block design was used. The experimental unit was a tree, and the same trees were used.

## 2.3.Pathogenicity.

Isolates of *Fusarium* species were tested for their ability to cause malformation by inculcation of mango healthy plants. Two sets of pathogenicity experiments were done in 2008 and 2009; the first set ; mango seedling cv. Seddek (two years old) was inoculated with  $10^5$  colony forming units of *Fusarium* spp either as apical buds injection or as inoculated soil. The second set seedlings of mango cvs. Timor, Seddek, Awes, Zebdia, El fonse, Fagar Kelan, Handi Besenra, Keet, Kant and Tomy were sown into soil inoculated with  $10^5$  colony forming units | g of soil inoculated with pathogenic fungi. For each experiment and tested isolate, four replications of six seedlings each were evaluated. Sterilized water was used as a control. Transplanted seedlings were monitored for development of malformation. At the end of the experiment (120 days), all surviving seedlings were examined for apical disease symptoms. Data were recorded on symptoms manifestation as diseases incidence and severity (from 1-4 scale ). Post inoculation colonization by Fusarium isolates in inoculated plants was determined by reisolation. Root, stem and malformed pieces, each approximately 5 mm in length, from each seedling, were surface sterilized in a 10% bleach solution (0.525% aqueous sodium hypochlorite; 1 part standard household bleach in 10 parts water), rinsed in sterile, distilled water, and placed on a selective agar medium for Fusarium spp. (Komada 1975). Plates were incubated under diurnal

cycles of cool, fluorescent light at about 24 <sup>o</sup>C fo2r 7-10 days. Emerging fungi were compared with inoculated isolates to determine whether they were the same morphological species.

## Statistical analysis.

The obtained data were statistically computed using the software SPSS for Windows (release 9.0.0, Dec. 18, 1998, standard version, SPSS Inc.). All treatments in the previous experiments consisted of three or four replicates.

## 3. Results and Discussion

## 3.1. Disease survey.

A disease survey in El-Behera, Giza and Ismailia Governorates was performed during the growing period as a preliminary study. Mean percentages of the disease incidence in cultivars were calculated on vegetative and blossom clusters (Table 1). Many isolates of Fusarium were obtained during routine isolations from seedlings, large trees exhibiting malformation disease symptoms. Data indicated that additional taxa were associated with MMD nine F. subglutinans, F. oxysporum, inculde Fsterilihyphosum, F. proliferatum, F. culmorum, F. nygamai, F. pseudonygamai, F.nelsonii and F. verticilioides. F. subglutinans proved high frequancy from all cultivars and location, ranging from 94.7to 84.5%. F. oxysporium, F. sterilihyphosum and F. proliferatum solates, exhibited moderate frequency, ranging from 11.5 to 1.56. The other isolates displayed very low in frequency from some cultivars, ranging from 0.23 to 0.76 %. Maximum infection of traditional cultivars was observed in Timour, Zebda, Awais and Alfonso. Exotic Tomy, Keet and Kent cultivars appeared to be the moderate infection varieties to all Fusarium spp. Mango malformation disease (MMD) is a serious disease in many areas where this important crop is grown. This disease causes abnormal development of vegetative shoots and inflorescences (Kumar et al 1993 and Yadav, 1999). Floral malformation is the most prominent symptom and is characterized by abnormal, thick and fleshy panicles (Kumar et al 1993). Affected panicles bear no fruit, resulting in significant economic losses (Kumar et al 1993 and Ploetz, 2003). Mango malformation disease was first described in India in 1891 (Marasas et al., 2006), and has since been shown to occur multiple location in Asia, Africa, and the Americas. Despite this fact, relatively little is known about the disease. The recent discovery that several Fusarium spp. are associated with MMD is intriguing. At least four taxa of Fusarium have been associated worldwide, with MMD including Fusarium subglutinans (previously F. mangiferae) in many growing regions, Fusarium sterilihyphosum in Brazil

and South Africa, and Fusarium sp. nov. and F. proliferatum (teleomorph: Gibberella intermedia) from Malaysia. To data, only F. subglutinans has been reported in Egypt (Ploetz et al., 2002). The origins of the associated Fusarium species are unknown. Thus, the present study were to determine the frequency of different fungi in malformed tissues and establish the cause of mango malformation in Egypt and determine of fungi associated with malformed tissues of mango on different traditional and exotic cultivars. Complete resistance has not been observed in anyone variety. Nine unique Fusarium spp. were isolated, identified and named F. Subglutinans, F. oxysporium, F. sterilihyphosum, F. proliferatum, F. culmorum, F. nygamai, F. pseudonygamai, F. nelsonii and F. verticilioides. We have routinely isolated high levels of F. Subglutinans from malformed disease mango trees . This species is most often isolated from the interior of stems, branches, and vegetative and blossom of trees displaying malformation symptoms. The infection frequency (within tissue infection) of F. subglutinans confirms its role in causation of malformation symptoms. However, Fusarium subglutinans appears to have a significant role in malformation. Symptoms of vegetative and floral malformation appeared where mycelium of Fusarium species were present in the tissue at high concentrations. Since, F. oxysporum, Fusarium sterilihyphosum and *F. proliferatum* solates, exhibited moderate frequency from most of cultivars and location. Fusarium subglutinans was commonly isolated from mango trees displaying malformation symptoms (Ploetz and Gregory, 1993, Freeman et al., 2004, Iqbal et al., 2006, Marasas et al., 2006). Reports that F. oxysporum Schlecht emend. Snyder & Hansen causes MMD indicate that a new, chlamydospore-producing taxon is involved (Marasas, et al., 2006). (Marasas, et al., 2006). Fusarium sterilihyphosum, on the other hand, has been isolated from malformed mango tissue in South Africa, meanwhile, F. proliferatum are associated with MMD in Malaysia (Marasas, et al., 2006 and Alvarado et al., 2006). Other numbers of Fusarium associated with either healthy or diseased plants are likely saprophytic; a much smaller number are capable of eliciting disease.

## 3.2. Disease progress.

Data in Fig. (1 and 2) indicated that symptoms of malformation were initially observed on January on vegetative stage and continues on blossom clusters of March and maximum symptoms was appeared in March and decline in May. Maximum disease severity was observed in the selected orchards. All tested cultivars were susceptible to infection with malformation expressed as disease progress. Traditional cultivars i.e. Al-Fonso, Timour, Awais and Zebda were the most susceptible cultivars, as they gave

the highest percentage of infection. Meanwhile, Dabsha and Dabsha were the moderate resistance. Data also show that in all exotic cultivars as Tomy, Keet and Kent appeared to be the least infection. In general, the highest disease incidence was found during the warm season February to April. Seasonal variations in the occurrence and severity of problem correlate with ambient temperature at flowering (Majumdar and Sinha, 1972). In Egypt panicles appearing on spring shoots are most severely affected (Shawky *et al.*, 1980). In Florida the heaviest infection occurs under unusually wet conditions (Campbell and Marlatt, 1986). The severity of the disease varies from variety to variety and tree to tree in the same variety.

## 3.3. Pathogenicty

Eight fungi viz. F. subglutinans, F. solani, F. oxyspoum, F. sterilihyphosum, F. proliferatum, F. moniliforme, F.avena and F.chlamydspore were tested using susceptible Seddek cultivar as apical injection or inoculated soil (Table 2). Data pertaining to artificial inoculations revealed that effort to produce disease by spores injection or soil inoculation. Soil inoculation was successful method. Four *Fusarium subglutinans* proved to be the dominant fungus with 100% sample's infection in inoculated soil. Fungi F. oxysporum, F. sterilihyphosum and F.proliferatum showed moderate infection in induced typical malformation symptoms in inoculated mango seedling and were re-isolated . Other *Fusarium* spp. give grown and root rots symptoms.

Four fungi viz. *F. subglutinans, F. oxysporum, F. sterilihyphosum* and *F. proliferatum* were elected for the other test study using eleven cultivars (Table 3). Significant differences (P=0. 05) were found among different isolates for the infection. *Fusarium subglutinans* proved to be the dominant fungus with 100% sample's infection in the seven local cultivars include Al-Fonso, Hindi Sennara, Seddek, Timour, Dabcha, Zebda, Ewais and Fagrkelan. Exotic keet, Tomy and kent appeared to be the moderate infection varieties giving 92.0, 96.0 and 93.7% tissue infection, respectively. Other fungi like *F. oxysporum* and *F. proliferatum* showed moderate infection level. One tested *F. sterilihyphosum* isolate was weakly virulent.

Data in Table 3 indicated that *F. subglutinans*, *F.oxysporum*, *F. sterilihyphosum* and *F. proliferatum* were found to be associated with malformed parts as well as colonized root stem. Maximum recovery (100%) was exhibited by *F. subglutinans* in all local cultivars include Al-Fonso, Hindi Sennara, Seddek, Timour, Dabcha, Zebda, Ewais and Fagrkelan. Moderate recovary was recorded in exotic keet, Tomy and kent .Other fungi like *F.oxysporum and F.*  *sterilihyphosum* showed moderate recovery level from either root or apical buds. *F. proliferatum* showed the least one. On healthy mango seedlings, a small conical apical bud gradually attaining its normal shape .

We planed to re-test these isolates in subsequent greenhouse inoculation trials to confirm their pathogenic behavior (Table 4). *Fusarium* isolates obtained from diseased mango trees, varied widely in their virulence on inoculated mango seedlings under greenhouse conditions. Isolate of *F. subglutinans* was pathogenic and the other tested isolate was moderately virulent on inoculated seedlings, that caused malformation on all cultivars.

In this study we have shown that at least four distinct Fusarium spp. are associated with mango malformation symptoms. F. subglutinans proved to be the dominant fungus infecting majority of the tissues. Fusarium mangiferae has been isolated from mango malformation symptoms in various geographical areas, such as South Africa, Florida, Egypt, India, Israel and Malaysia (Ploetz et al., 2002 and Kvas et al., 2008). The infection frequency and disease incidence of other fungi remained much less. Complex Strains of F. subglutinans, F. oxysporum, F. sterilihyphosum and F. proliferatum induced typical malformation symptoms on mango seedlings and trees in Egypt . Also, this results of this study, together with those of Steenkamp et al (2000), have shown that mango malformation in South Africa is associated with two distinct species, F. subglutinans var mangiferae and F. sterilihyphosum. The pathogenic interaction with floral buds resulted in high incidences of malformation which started early in the floral season, extended up to February and reestablished in July. The results of these studies will be helpful for future statistics, management, forecasting and experimental designing.

## Acknowledgments

This manuscript funded from the project "New applied approaches to promote productivity and Quality of some fruit crops (Mango)" National Research Centre, 2007 to 2010.

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	El-Bohera		Giza		Ismalia		
Cultivars	Fungi isolated	(%) Infection frequency	Fungi isolated	(%) Infection frequency	Fungi isolated	(%) Infection frequency	
Hindi Sennara	F. subglutinans F. oxysporum F. sterilihyphosum F. proliferatum F. culmorum F. nygamai F. pseudonygamai	84.7 6.80 3.52 2.43 0.34 0.46 0.25	F. subglutinans F. oxysporum F. sterilihyphosum F.proliferatum F. culmorum F.nygamai F.pseudonygamai	88.5 5.43 3.72 3.23 0.34 0.36 0.36	F. subglutinans F.oxysporum F. sterilihyphosum F.proliferatum F. culmorum	88.4 4.06 3.33 2.35 0.37	
El- Founso	F. subglutinans F. sterilihyphosum F. proliferatum F.oxysporum F.nygamai F.pseudonygamai	89.4 4.42 3.34 2.54 0.30 0.54	ND*		F. subglutinans F.oxysporum F.proliferatum F. sterilihyphosum	90.0 4.24 3.23 2.54	
Sadeka	F. subglutinans F.proliferatum F.oxysporum F.nygamai F.nelsonii	88.5 7.76 3.43 0.32 0.54	F. subglutinans F.oxysporum F.proliferatum F.nygamai	87.5 8.87 3.34 0.98	F. subglutinans F.oxysporum F.nygamai F.ndsonii	87.7 6.87 0.65 0.13	
Timour	F. subglutinans F.oxysporum F.nygamai F. sterilihyphosum F.proliferatum F. culmorum	91.7 2.43 0.67 1.56 1.56 0.45	F. subglutinans F.oxysporum F.nygamai F. sterilihyphosum F.nelsonii	93.6 2.65 0.76 1.65 0.76	F. subglutinans F.oxysporum F.nygamai F. culmorum F. proliferatum F. sterilihyphosum F.ndsonii	93.6 2.54 0.34 0.65 2.65 1.87 0.23	
Dabcha	F. subglutinans F. proliferatum F.nygamai F.acuminatum	94.8 4.76 0.54 0.27	F. subglutinans F. proliferatum F.nygamai F.oxsporum	92.6 4.87 3.53 0.76	F. subglutinans F.nygamai	95.8 0.65	
Zebda	F. subglutinans F.oxysporum F. proliferatum F. sterilihyphosum	91.8 3.54 2.43 2.43	F. subglutinans F.oxysporium F. proliferatum F. sterilihyphosum	92.4 4.34 2.42 2.56	F. subglutinans F.oxysporium F. proliferatum F. sterilihyphosum F.verticilioides	91.3 2.43 2.54 2.54 0.24	
Ewais	F. subglutinans F. sterilihyphosum F.oxysporum F.proliferatum F.nygamai	91.5 3.66 3.33 2.66 0.75	F. subglutinans F.pseudonygamai F. sterilihyphosum F.nygamai F.nelsonii	94.4 2.34 3.54 0.54 0.43	F. subglutinans F.oxysporum F. sterilihyphosum F.proliferatum F.verticilioides	91.3 4.32 3.43 3.12 0.32	
Fagrkela n	F. subglutinans F.oxysporium F.nygamai	93.7 5.65 0.46	F. subglutinans F.oxysporum F.nygamai	94.7 5.76 0.48	F. subglutinans F.oxysporium F.nygamai	94.6 4.76 0.45	
Keet	F. subglutinans F. sterilihyphosum F. proliferatum F.nygamai	88.5 5.0 5.5 0.54	ND		F. subglutinans F. sterilihyphosum F. proliferatum F.nygamai	89.1 6.9 5.5 0.23	
Tomy	F. subglutinans F. proliferatum F.nelsonii	84.5 10.5 0.32	ND		F. subglutinans F.proliferatum F.ndsonii	88.5 11.5 0.24	

Table 1. Fungi associated with malformed	parts of mango in Egypt
Table 1. Fully associated with manormed	parts of mango in Egypt.

\*ND: Not detected



Figure 1. Mango malformation on blossom clusters.

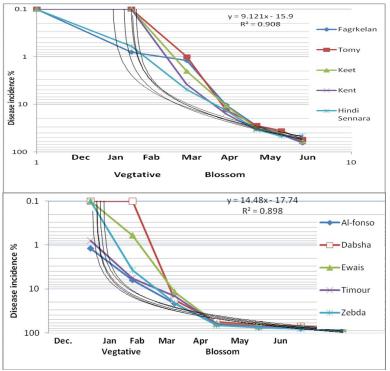


Figure 2. Disease progress curves of malformation in vegetative and floral shoots of mango in El Bohera Governorate

Scour rigs								
Treatment	Infested	l soil	Injection buds					
	Disease incidence %	Disease severity	Disease incidence %	Disease severity				
F. subglutinans	100.0	4.0	75.0	3.3				
F. solani	0.0	0.0	0.0	0.0				
F. oxyspoum	50.0	1.3	25.0	0.3				
F. sterilihyphosum	50.0	2.3	25.0	2.0				
F. proliferatum	50.0	1.6	25.0	1.3				
F.moniliforme	0.0	0.0	0.0	0.0				
F.avena	0.0	0.0	0.0	0.0				
F.chlamydspore	0.0	0.0	0.0	0.0				
LSD	25.0	0.5.	12.0	0.5				

 Table 2. Comparative virulence of selected Fusarium isolates on inoculated mango cv. Seddek seedlings

 Table 3. Comparative virulence of selected *Fusarium* isolates on inoculated mango cultivars seedlings

Cultivars	% Infection						
	F. subglutinans	F. oxyspoum	F. sterilihyphosum	F. proliferatum			
Hindi Sennara	100.0	4.56	1.76	3.76	27.5		
Seddek	100.0	9.58	3.87	8.56	30.5		
Timour	100.0	11.4	5.87	9.54	31.7		
Dabcha	100.0	8.54	6.87	6.34	30.4		
Zebda	100.0	13.7	8.56	11.7	33.4		
Ewais	100.0	10.5	7.26	9.65	31.1		
Fagrkelan	100.0	8.08	5.26	7.98	30.3		
Al Fonso	100.0	13.5	9.65	9.45	34.6		
Keet	90.0	2.61	1.34	1.65	23.9		
Tomy	96.0	0.94	0.00	0.00	24.2		
Kent	93.7	0.75	0.00	0.00	23.6		
Mean	97.9	7.96	4.77	5.91			
LSD	2.43	2.95	2.54	2.76			

Table 4. Percent recovery of Fusarium sp. from inoculated mango cultivars.

Cultivars								
	F. subglutinans		F.oxysporum		F. sterilihyphosum		F. proliferatum	
	Root colonized	Apical colonized	Root colonized	Apical colonized	Root colonized	Apical colonized	Root colonized	Apical colonized
Hindi Sennara	100.0	100.0	2.36	3.65	2.06	1.26	3.53	2.13
Seddek	100.0	100.0	5.27	6.76	5.26	4.27	4.08	3.00
Timour	100.0	100.0	6.07	5.43	8.24	5.00	5.04	2.04
Dabcha	100.0	100.0	8.07	6.76	4.04	6.17	4.24	2.24
Zebda	100.0	100.0	6.96	5.43	7.17	4.66	3.17	2.11
Ewais	100.0	100.0	6.46	8.45	7.55	3.26	3.25	2.25
Fagrkelan	100.0	100.0	4.74	6.87	4.58	3.64	1.08	0.58
Al Fonso	100.0	100.0	7.54	6.98	6.98	5.34	3.87	2.43
Keet	66.6	80.0	1.04	1.70	0.75	0.95	0.90	0.34
Tomy	63.0	76.0	1.95	1.45	0.50	0.45	0.41	0.64
Kent	83.7	80.0	1.75	1.65	0.84	0.95	0.50	0.45
LSD	3.67	4.23	0.89	0.95	0.92	0.78	0.75	0.45

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