Role of Cultivars, Growth Regulators and Biocides on the Incidence of Mango Malformation in Egypt

Azza M. K. Azmy
Plant Pathol. Res. Instit., ARC, Giza , Egypt

Abstract— Isolation trials from malformed mango inflorescences frequenty yielded Fusaraium mangiferae .Seven local mango cultivars, i.e. Ewasy, Fagri klan, Hendy , Hendy Sennara , Keitt, Kent, and Saddeka of 10 years old were evaluated for their ability to the natural infected by malformation disease during 2012 growing season. Data revealed that Saddeka cv. followed by Ewasy cv. were the most susceptible ones to the disease incidence and Keitt was the lowest affected one. The effect of three commercial growth regulators, i.e. Agrotone (NAA), Berelex (GA3), Cultar (paclobutrazol) and four biocides, i.e. AQ10 (Ampelomyces quisqualis), Bio-ARC (Bacillus megaterium) , Bio-Zeid (Trichoderma album) and Blight Stop (Trichoderma spp.), on the incidence of mango malformation was evaluated in the field under the natural infection by the disease during 2012 /2013 and 2013/ 2014 growing seasons. Results indicated that the two growth regulators ,i.e. Cultar and Agrotone and the biocide Bio-Zeid were the most effective treatments in reducing the disease and increasing the produced fruit yield than the other treatments when each of them was used alone. However, the combination treatment between the growth regulator Cultar and the biocide Bio-Zeid was the most efficient treatment in this regard.

Key words : Mango malformation , biocides , disease incidence , Fusarium mangiferae ,growth regulators, varietal reaction .

1 INTRODUCTION

Mango (Mangifera indica L.) Fam. Anacardiaceae is one of the most important and favorite fruit in Egypt for local consumption and exportation . The cultivated area reached about 265 350 feddan until 2014, which produced about 927352 ton with an average of 4.4 ton/ feddan (Anonymous, 2014).

Under Egyptian conditions, mango trees are suffered from infection by many pests ,i.e. bacterial, fungal , phytoplasma and viral diseases in addition to physiological disorder and mites (El-Banna and El-Deeb, 2001 ; Ansari et al., 2008 and 2013; Youssef et al.,2009 and Haggag, 2010 and Haggag et al.,2014). However, malformation which causes gross deformations of vegetative and floral tissues in Mangifera indica, is one of the most threaten disease, which fungi , mites , phytoplalsma and viruses , physiological factors , ethylene , cyanide , malformins and mangiferins were reported as the causal of this phenomena (Singh and Dhillon, 1992 ; El-Banna and El-Deeb, 2001; Tapan et al.,2006 and Ansari et al., 2008 and 2013).In addition, panicles appearing on spring shoots are most severely affected (Shawky et al., 1980 and Youssef et al.,2009).

Mahrous (2004) reported that mango trees sprayed with NAA, IBA, phosphoric acid and GA3 showed great decrease in the incidence of floral malformation disease in the next flowering season compared with the untreated trees. Also, fruit yield from treated trees was significantly higher during the first and the second seasons and NAA gave the best results followed by IBA and GA3 .

Mango malformation and spongy tissue continue to be serious maladies though some practical and effective measures have been recommended and the use of paclobutrazol (Cultar) has become common , where one spray of 50 ppm GA3 has been useful to prevent recurrent flowering (Gunjate, 2009).

Exogenous application of NAA by 200 ppm and deblossoming at bud burst stage was found to be the most effective treatment in reducing the incidence of floral malformation and improving the fruit yield and quality of mango more than NAA application alone without deblossoming or shoot and stalk pruning (Mohan and Prakash, 2011).

Soil drenching with paclobutrazol was significantly effective in suppressing vegetative growth and significant differences were recorded in all treated cultivars of mango as compared to control regarding the emergence of reproductive shoots, fruit setting, panicle length, fruit drop, intensity emergence of malformed panicles, reducing malformation and increased mango yield (Nafees et al., 2010). Mango growers spray 200 ppm NAA during the period of flower bud differentiation ,which reduced the rate of fruit malformation (Tingchao et al., 2009).In addition, trees treated with paclobutrazol showed the lowest level of floral malformation at 5 and 10 g. a.i. per tree as soil drench compared with NAA at 100 and 200 ppm as spraying the tree. However, paclobutrazol applied to the mango trees either as a foliar spray or as a soil drench at 10-60 g. a.i. / tree prior to flower bud differentiation during the first week of Oct. reduced the incidence of floral malformation and enhanced yields and fruit quality compared with untreated controls. Soil application was usually the more effective and the best result with rate 20 gram a.i./tree (Singh and Dhillon,1992 ;Singh et al., 2004 and Gunjate, 2009) .

The bioagents Trichoderma veredi , T. virens and T. harzianum were evaluated in culture with the pathogen Fusarium to monitoring their antagonistic effect . It was

found that all the three isolates of bioagents significantly checked the growth of the fungus Fusarium. The best results was obtained with T. harzianum followed by T. virens and T. veredi, a similar result was also observed in the case of culture filtrates of Trichoderma spp. (Kumar et al., 2012). Also, Korsten et. al. (1992) found that Bacillus licheniformis and B. subtilis reduced mango diseases when evaluated in preliminary field spray application in south Africa for controlling mango diseases.

2 MATERIALS AND METHODS

Isolation , purification and identification of the associated fungus :

Naturally malformed mango samples were collected from El-Adleia district, Belbees county , El-Sharkia governorate during 2012 growing season. The collected samples washed thoroughly and cut into small pieces then surfaces sterilized by immersing into 2.0% sodium hypochlorite solution for two minutes , rinsed three times in sterilized water and dried samples placed on PDA medium containing 1000 ppm of streptomycin and incubated at 25°C for one week. The emerged fungus was picked up , purifed using single spore techniques as mentioned by Dhingra and Sinclair (1985) and identified based on it' s morphological and cultural characters and the description of Booth(1971). Identification was confirmed by Mycological Center , Fac. of Sci., Assuit Univ. Assuit, Egypt.

Field experiments :

Field experimrnts were conducted during 2012/2013 and 2013/2014 growing seasons on 10- years old of mango trees at El Adleia disrtrict , Belbees county, El Sharkia governorate. All agriculture practices of irrigation, fertilization , pests and weed management , pruning and the other cultural practices for mango trees were carried out as recommended by Min. of Agric. and Land Reclamation..

Varietal reaction :

Seven mango cultivars, i.e. Ewasy , Fagri Klan, Hendy, Hendy Sinnara , Keitt, Kent and Saddeka were evaluated to their susceptibility to the natural infection by malformation disease during 2012 growing season . Three trees for each cv. were carefully examined as a replicate to assess the natural infection by malformation as mentioned under diseaase assessment and three replicates were used for each cultivar and the average of disease incidence was recorded.

Effect of growth regulators and biocides:

Three commercial growth regulators, i.e. Agrotone (NAA) , Cultar (paclobutrazole) , Berelex (GA_3) and four biocides, i.e. AQ10 (Ampelomyces quisqualis) , Bio-ARC (Bacillus megaterium). Bio-Zeid (Trichoderma album) and Blight Stop (Trichoderma spp.) at the recommended doses as shown in Table (1) were sprayed, with exception of Cultar , which was used as soil drench. Triton (B 1956) was added to all sprayed materials .

The aforementioned materials were used to evaluate their effect on decreasing the incidence of malformation disease on Saddeka cv. (the highest susceptible cv.) of 10 years old. Growth regulators were applied twice; the first treatment was done in 15th Oct.of each seaon after pruning dry shoots and stalk soon after harvest, prior of flower bud differentiation and the second treatment in 15 th Febr. of each seanon at bud burst stage after deblossoming and removing early flowers and all affected inflorescences with three additional nodes (20 cm.) behind (Mohan and Prakash, 2011). Spraying of the biociddes, The first and the second were, also, sprayed twice at the aforementioned times throughout the two seasons.

Table (1) : The tested growth regulators and biocides :

Trade name	Active ingredient or/bioagent	Dose / 100 L. water
Agrotone	Naphthalien acetic acid (NAA)	200 ppm (60g / 100 L.water)
Cultar	Paclobutraozol	(20 g a.i. /tree)
Berelex	Gibbereilic acid (GA_3)	50 ppm 5 tablets of Berelex
AQ 10	Ampelomyces quisqualis	5 g
Bio-ARC (local biocide)	Bacillus megaterium	250 g
Bio-Zeid (local biocide)	Trichoderma album	250 g
BLight Stop (local biocide)	Trichoderma spp.	1 liter
Triton (B 1956)	Distributed substance	25 ml

The combined treatment between Cultar and Bio-Zeid was also done , which they were of high efficency in reducing mango malformation (unpublished data). The treatment was carried out , which the biocide Bio-Zeid was sprayed two times in 15 th Oct. and in 15 th Febr. as mentioned befor and Cultar was added as soil drench at 20 g. a.i. / tree in 15 th Oct and 15 th Febr. of each seanon.

Disease assessment :

Disease incidence was estimated by counting all of the tested inflorescences and recorded the normal and malformed inflorescences per every tree then disease incidence was calculated according to the following formula (Singh and Dhillon ,1990):

$$\% \text{ Disease incidence} = \frac{\text{Number of malformed inflorescences}}{\text{Total number of the tested inflorescences}} \times 100$$

Efficiency of the sprayed treatments

Efficiency was determined according to the following formula (Ghoneim ,1991):

$$\% \text{ Efficiency} = \frac{\text{Infection in control} - \text{infection in treatment}}{\text{Infection in control}} \times 100$$

-Fruit yield for each tree:

The produced fruit yield of each tree was havested periodically , weighed and the average weight of fruits in kg. per tree was recorded.

Statistical analysis:

Data were statistically analyzed and treatments were determined according to Duncan's multiple range test (Duncan, 1955).

3 RESULTS

Isolation, purification and identification of the responsible fungus of malformation:

Isolation trials from naturally malformed samples of Saddeka mango cv. showing typical symptoms of mango malformation (Fig.1) were carried out during 2012 growing season. Isolation trials yielded the fungus Fusarium mangiferae, which was frequently associated with mango malformation as similer to which known from the published facts and researches .

Fig.(1). Mango tree showing typical symptoms of inflorescences malformation.

Varietal reaction :

Data presented in Table (2) indicate that all the inspected cultivars, i.e. Ewasy , Fagri Klan, Hendy, Hendy Sinnara, Keitt, Kent and Saddeka to the natural infection by malformation were susceptible to the natural infection by malformation with different degrees of disease incidence . Saddeka cv. was the most susceptible one followed by Ewasy cv. being 31.6 and 30.8%, respectively.Whereas, Keitt and Fagri Klan cvs. showed the lowest susceptibility being 5.3 and 7.4%, respectively. The rest cultivars were of moderate susceptibility.

Table (2) . Susceptibility of some mango caltivars to the natural infection by malformation disease during 2012 growing season at El-Sharkia governorate.

Cultivars	% Disease incidence
Fagri Klan	7.4[b]
Kent	10.1[c]
Keitt	5.3[a]
Hendy Sinnara	26.7[e]
Hendy	23.7[d]
Saddeka	31.6f
Ewasy	30.8f

Duncan multiple range significant at Alpha (0.05).

Means with the same letter are not significantly different.a,b,c., values in the same column with different superscripts differed significantly.

Effect of growth regulators and biocides on the incidence of the natural infection by malformation disease and fruit yield during 2012/2013 and 2013/2014 growing seasons :

Data presented in Tables (3 and 4) show the effect of treating mango trees (Saddeka cv.) by three commercial growth regulators, i.e. Agrotone, Berelex and Cultar and four biocides, i.e. AQ10 , Bio-ARC, Bio-Zeid and Blight Stop each alone as well as the combination between the growth regulator Cultar and the biocide Bio-Zeid under shoot, stalk pruning and deblossoming compared with untreated control, and untreated but with pruning and deblossoming control, on management of the natural malformed trees during 2012/2013 and 2013/2014 growing seasons.

Table (3) indicates that all the tested treatments significantly reduced the incidence of mango malformation with significant increase in the produced fruit yield. Also, the untreated but pruned and deblossomed trees, showed considerable decreased in the incidence of malformation with an increase in the fruit yield, being 21.7 % disease incidence and 46.7 kg. fruits / tree compared with untreated control, being 31.3 % and 38.7kg. fruits / tree. However, the combination between Cultar (Paclobutrazol) and Bio-Zeid was the best treatment in reducing malformation incidence being 86.3% efficiency and 87.3 kg. fruits /tree followed by Cultar, being 74.4% and 83.7 kg. fruits / tree, which caused the best effect as a growth regulator followed by Agrotone (NAA), being 71.2% efficiency and 78.3 kg. fruits/ tree . Meanwhile, Berelex (GA3) was the lowest effective one, being 68.1% efficiency but with good yield 81.3 kg. fruits / tree. However, biocides showed moderate effect on disease incidence and fruit yield .In this regard, Bio-Zeid was the most effective one, being 60.7% efficiency and 77.3 kg. fruits / tree followed by Blight Stop and AQ10 ,being 59.4 and 70.7% and 58.5 efficiency and 66.3 kg. fruits / tree, respectively. Whereas, Bio-ARC was the lowest effective bioicide, being 56.2% efficiency and 55.7 kg. fruits / tree.

Table (3) . Effect of some commercial growth regulators and biocides in reducing the incidence of malformation disease (Saddeka cv.) in the open field during 2012/2013 growing season at El-Sharkia governorate.

Treatments	% Disease incidence	% Efficiency	Average fruit yield Kg/tree
Agrotone	9.0[de]	71.2	78.3[c]
Berelex	10.0[d]	68.1	81.3[b]
Cultar	8.0[e]	74.4	83.7[b]
AQ 10	13.0[c]	58.5	66.3[e]
Bio-ARC	13.7[c]	56.2	55.7[f]
Bio-Zeid	12.3[c]	60.7	77.3[c]
Blight Stop	12.7[c]	59.4	70.7[d]
Combination between Cultar +Bio-Zeid	4.3[f]	86.3	87.3[a]
Control*	21.7[b]	--	46.7[g]
Control**	31.3[a]	---	38.7[h]

Duncan multiple range significant at Alpha (0.05).Means with the same letter are not significantly different.a,b,c., values in the same column with different superscripts differed significantly.

* Untreated with any of the tested materials but pruned and deblossomed.

** Untreated with any of the tested materials aswell as un- pruned and un-deblossomed.

Table (4) reveals that all the tested treatments in the second seasons 2013/2014 showed the same trend of the obtained data in the frist seasons of 2012/2013 with some incresae in the values of the efficiency of the tested compounds and the produced fruit yield.

Table (4) . Effect of some commercial growth regulators and biocides in reducing the incidence of malformation disease (Saddeka cv.) in the open field during 2013/2014 growing season at El-Sharkia governorate.

Treatments	% Disease incidence	% Efficiency	Average fruit yield Kg. /tree
Agrotone	8.3[e]	74.1	75.7[d]
Berelex	9[e]	71.9	80.7[c]
Cultar	7.3[e]	77.2	85.7[b]
AQ 10	11.7[d]	63.4	65.3[f]
Bio-ARC	14.0[c]	56.3	58.3
Bio-Zeid	11.0[d]	65.6	80.3[c]
Blight Stop	11.3[d]	64.7	72.3[e]
Combination between Cultar +Bio-Zeid	4.0[f]	87.5	88.3[a]
Control*	23.3[b]	---	44.3[g]
Control**	32.0[a]	---	36.7[h]

Duncan multiple range significant at Alpha (0.05).Means with the same letter are not significantly different.a,b,c., values in the same column with different superscripts differed significantly.

* Untreated with any of the tested materials but pruned and deblossomed.

** Untreated with any of the tested materials aswell as un-pruned and un-deblossomed.

4 DISCUSSION

This study was carried to find out a method or trial to management or lowering the infection by mango malformation in order to increase the planted area with increasing the economic income to the growers .

The most important and damaging symptom on mangoes is the development of malformed or abnormal inflorescences. This is caused by hormonal imbalance associated with the Fusarium infection (F. mangiferae) leading to the development of sterile florets on short internodes and there is no fruit production. Vegetative symptoms include distorted shoots with shortened internodes and often with a witches broom appearance that are most common in younger trees. Cultural practices e.g. pruning malformed flowers, avoiding damage to tree shoots would definitely contribute to prevent the spread of the causal pathogen throughout the orchards (Kvas et al., 2008).

F. mangiferae was found to be associated with mango malformation . Hormonal imbalance, most probably ethylene , might be responsible for deformed functional morphology of panicle. Further, a signal transduction mechanism of stress stimulated ethylene imbalance causing physio- morphological changes in reproductive organs of mango flower and thereby failure of fertilization and fruit set (Ansari et al., 2013). Two types of mango malformation occur, vegetative and floral. Etiology and control of mango malformation are yet not well understood. Fungi , mites , phtoplasma , viruses , physiological factors, ethylene, malformins and mangiferins are its reported causes (El-Banna and El-Deeb, 2001 ; Ansari et al., 2008 and 2013; Youssef et al.,2009 and Haggag, 2010 and Haggag et al.,2014).

Mango malformation is involved by malformin–like substances, with malformin stimulating ethylene production causing a hormonal imbalance such higher levels of abscisic acid and zeatin but lower levels of IAA and gibberellic acid causing disturbed metabolism inducing mal formation consequently decreasing mango yield and quality (Zora Singh, 2000).

Malformation is not merely a serious threat but a menace to the mango industry in several mango growing areas of the world, produces abnormal vegetative shoots and inflorescence which do not bear fruits causing heavy losses in yield. Therefore, an incite was made to minimize the menace by the foliar application of different growth regulators ,which increased the leaf index, length of flower panicles , fruit set , and reduction of malformation (Rajput et al., 2013).

Endogenous ethylene levels in mango tissues were found to be higher in malformed tissues than the respective healthy tissues at different developmental stages of flowering in mango (Britz et al., 2002 ; Krishnan et al.,2009 and Rymbai and Rajesh (2011) and Fusarium spp. are capable of producing ethylene. However, the role of ethylene induction by F. mangiferae in causing mango malformation has not been successfully demonstrated. The obtained data by Rymbai and Rajesh (2011) suggest that F. mangiferae could contribute to the malformation of mango by producing ethylene and stimulating stress ethylene, production in malformed tissue of mango. The disease severity is reflected with the mean temperature preceding flowering. It is most severe where mean temperature remains between 10–15°C. It is mild where the corresponding temperature is 15–20°C, sporadic at 20–25°C and nil over 25°C.

The obtained data revealed that both Saddeka and Ewasy cvs. were the most susceptible one to mango malformation and Keitt followed by Fagri Klan were the lowest affected ones. In general,planting the resistant cvs. to plant disease is of great important, but in most cases such resistant cvs. are contingent by the consumer taste , high productivity , appropriate local environment conditions ... ect. Many authors used the resistant cvs. as a trial for lowering the infection by malformation but with another trials as IPM (Badliya 1990 ; Azzouz et al., 1984 and Rymbai and Rajesh ,2011).

It has been found that the three tested commercial growth regulators i.e., Agrotone (NAA), Berelex (GA3) , Cultar (paclobutrazol), and the four tested biocides, i.e. AQ10, BioArc, Bio-Zeid and Blight Stop and comination btween Cultar and Bio-Zeid were evaluated for their efficiency on management of mango malformation under field condition during 2012 and 2013 growing seasons at El-Sharkia governorate . Data observed that treated mango Saddeka cv. with any of the tested treatments significantly reduced the incidience of malformation disease. The combination between Cultar as growth regulator and the biocides Bio-Zeid was the best treatment in reducing the disease and increasing the fruit yield during 2012 and 2013 growing season.

All the tested growth regulators and bioagentss significantly reduced the incidence of mango malformation with significant increase in the produced fruit yield. Also, the untreated but pruned and deblossomed trees, showed considerable decreased in the incidence of malformation with an increase in the fruit yield compared with untreated control. However, the combination between Cultar (paclobutrazol) and Bio-Zeid was the best treatment in reducing malformation incidence followed by Cultar then by Agrotone (NAA). Meanwhile, Berelex (GA3) was the lowest effective one and

biocides showed moderate effect on disease incidence and fruit yield. However, Bio-Zeid was the most effective biocide followed by Blight Stop and AQ10. Whereas, Bio-ARC was the lowest effective biocide. The obtained results are in agreement with those reported by (Korsten et al., 1992 ;Kumar et al., 2012)

Mahrous (2004) reported that mango trees sprayed with NAA, IBA, phosphoric acid and GA3 showed great decrease in the incidence of floral malformation disease in the next flowering season compared with the untreated trees. Also, fruit yield from treated trees was significantly higher during the first and the second seasons and NAA gave the best results followed by IBA and GA3 .

Mango malformation and spongy tissue continue to be serious maladies though some practical and effective measures have been recommended and the use of paclobutrazol (Cultar) has become common as spray or soil drench (Gunjate, 2009 and Nafees et al., 2010). Also, exogenous application of NAA by 200 ppm and deblossoming at bud burst stage was found to be the most effective treatment in reducing the incidence of floral malformation and improving the fruit yield and quality of mango more than NAA application alone without deblossoming or shoot and stalk pruning (Singh et al., 2004 ; Gunjate, 2009 ;Mohan and Prakash, 2011 and Tingchao et al., 2009).

The management strategies of keeping orchards clean could be achive by planting lowest affect cultivars such as Kiett and Fagri Klan cvs. as well as using commercial growth regulators and biocides in order to reducing the incidence of mango malformation. These methods must be carried out under role of pruning of dry shoots and stalk prior of bud differentiation and deblossoming by removing early flowers at bud burst in the beginning of the season.

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