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An overview on mango malformation and the potential approaches to their management

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Abstract
Mango (*Mangifera indica* L.) is the fifth largest cultivated fruit crop globally with the yields of approximately 40 million tonnes, second only to banana among the tropical fruit species (FAOSTAT, 2015). Mango malformation (MMD) is an ambiguous disease of mango with the tremendous economic importance throughout the mango growing regions. Initially, research works were focused over finding the causes of this malady and different microorganism were isolated and claimed to be its causal agent. Despite not killing the host plant, it affects the leaf panicles and inflorescence leading to the massive reduction of yield. The most effective management of disease includes the avoidance of inoculums, selection of resistant varieties and the potential control of disease are targeted to eradicate the causative agent. Physical alteration followed by chemical treatment like Prochloraz and benomyl spray results in the reduction of disease incidence and increment of yield. Strong antifungal concoction derived from different plant extract was reported to be beneficial. As the malformed mango lacks some PGRs compared to healthy plants, researchers found the spray of the PGRs to be useful to increase the yield by reducing disease incidence. Different bacteria are found to secrete the antagonist substance capable of suppressing the growth of fungi related to this disease and many pathologists have been working to tackle the disease incidence but none of the findings are 100% effective and the disease still remains as a mystery. Hence, the present review aims to provide the brief information of the disease and effective management strategies.

Keywords: Mango malformation, causes, symptoms, management

Introduction
Mango (*Mangifera indica* L.) is one of the most important fruit crops of India and belongs to family Anacardiaceae. It is regarded as the king of fruits in India (Purseglove, 1972) [18] where it has been cultivated for at least 4,000 years and has great cultural and religious significance (Popenoe, W. 1932 and Purseglove, J. W. 1972) [17, 18]. It is the fifth largest cultivated fruit crop globally with yields of approximately 40 million tonnes, second only to banana among the tropical fruit species (Faostat, 2015) [3]. Mango cultivation originated in India and expanded throughout Southeast Asia (Mukehrjee & Litz, 2011) [12]. Mango is a complicated tree and it is always confusing to understand its pattern of vegetative and reproductive growth and its various disorders.

Mango malformation is one of the most important and destructive diseases of this crop worldwide (Ploetz & R. C. 2001) [15]. Watt first reported it in 1891 in Darbhanga, Bihar, India. Now has spread elsewhere in Asia in other countries viz. India, Egypt, South Africa, Brazil, Sudan, USA, Israel, Mexico, Bangladesh, and Pakistan. Mango cultivation in many countries worldwide is severely affected by mango malformation disease (MMD) (Kumar *et al*., 1993; Ploetz & Freeman, 2009) [9, 15]. It affects inflorescences and vegetative portions of the plant. Although trees are not killed, the vegetative phase of the disease impedes canopy development and the floral phase reduces fruit yield dramatically; substantial economic losses can occur since malformed inflorescences do not bear fruit. The malformation is an intricate disorder of mango and causes severe yield losses since malformed inflorescences do not bear fruit. The losses extend from 50 to 60%, in case of severity the loss may be up to 100% (Summanwar, 1967) [22].

Causes
Epidemiology of mango malformation was not well understood (Kumar *et al*., 1993; Kumar *et al*., 1993; Ploetz *et al*., 2001) [8, 10, 15]. However, many studies have proven that *Fusarium mangiferae* is the pathogen responsible for mango malformation disease and Koch's postulates have been completed successfully with this fungus in various countries worldwide (Kumar *et al*., 1993) [10]. Although the cause of malformation has been controversial, but fungus is one of the major possibility causes.

Summanwar *et al.* (1966) [33] and Varma *et al.* (1969) [31, 50, 51, 52] in India were the first to report that the floral and vegetative malformation in mango was caused by *Fusarium moniliforme* (recognized later as *F. subglutinans*). The disease has been associated with physiologic disorders and hormonal imbalances (Iyer *et al*., 2009 and kumar *et al*. 1991) [32] and attacks of an *Eriophyid mite, Aceria mangiferae*, has been attributed to the cause of mango malformation, however, certain studies indicate that the mite may only play a role in wounding and transfer of the fungal pathogen to and from infection sites (Gamliel-Atinsky *et al*., 2009a; Ploetz *et al*., 1994; Kumar *et al*., 1993) [58, 10, 34]. In recent years, additional *Fusarium* species such as *F. sterilihyphosum* from Brazil and South Africa, *F. mexicanum* from Mexico, *F. proliferatum* from China and most recently, *F. tupiense* from Brazil, were implicated in malformation (Britz *et al*., 2002; Lima *et al*., 2008, 2012; Marasas *et al*., 2006; Newman *et al*., 2012; Otero-Colina *et al*., 2010) [35, 37, 38, 39, 40]. However, Koch's postulates have only been completed for *Fusarium subglutinans* and *F. oxysporum* as the causal agents of malformation.

Epidemiological studies on the malformation of mango are limited; however, temperature apparently has a key role in disease development. In India, the disease is present in all mango-producing areas (Verma *et al*., 1971) [25, 26], with a lower incidence in the southern and eastern than in the northern region. Temperatures in those regions are warmer than in the north, where cold conditions precede flowering. Earlier emerging floral buds are the most severely damaged, whereas later ones escape the disease (Kumar *et al*., 1993) [9]. Escape was attributed to the occurrence of relatively high temperature during panicle development. Coincidentally, a study of seasonal variation of the population density of *F. moniliforme* on mango shoots indicated that spore density reached a maximum in February, when temperature ranged from 8 to 27°C and the humidity was 85%, and that a decline of spore density coincided with hot, dry conditions (Kumar *et al*., 1993) [9].

Today, it is well cited and confirmed that a fungus *Fusarium moniliforme* (*Gibberella fujikuroi*) var. subglutinans is the dominant causal agent of mango malformation (Campbell and Marlatt, 1986; Salazar- Garcia, 1995; and Kumar *et al*., 1997, Ploetz and Gregory, 1993 and Britz *et al*., 2002) [35]. This fungus was subsequently referred to as *F. subglutinans.* However, *F. subglutinans* sensu lato is a very large and polyphyletic species complex that contains several host-specific taxa that cause a number of different plant diseases including ear rot of maize, pokkah boeng disease of sugarcane, pitch canker of pines, fusariosis of pineapple and malformation disease of mango (Steenkamp *et al*., 2000) [41]. Total confusion resulted for many years because the fungi that cause this array of different plant diseases, including mango malformation disease, were all called "*Fusarium subglutinans*". *Fusarium mangiferae* has been identified in China, Egypt, India, Israel, Malaysia, Oman, South Africa, Spain, Sri Lanka and the USA, and appears to be the most common causal agent of MMD worldwide (Freeman *et al*., 2014c) [5]. A second MMD causal agent, *F. sterilihyphosum*, was described I South Africa (Britz *et al*., 2002) [35] and Brazil (Lima *et al*. 2009) [42], while another causal agent, F. mexicanum, was described exclusively from Mexico (Otero-Colina *et al*., 2010) [40]. A fourth recently described species, *F. tupiense* sp. Nov. (Resembling *F. sterilihyphosum*), has been shown to cause malformation in Brazil (Lima *et al*., 2012) [36], Senegal (Senghor *et al*., 2012) [43] and Spain (Crespo *et al*., 2016) [44]. Most recently, *F. pseudocircinatum* has been described as an additional MMD causal agent in Mexico and the Dominican Republic (Freeman *et al*., 2014b; Garc_ıa- L_opez *et al*., 2016) [45, 63]. In addition, F. mangiferae, *F. proliferatum*, *F. pseudocircinatum* and other *Fusarium* species have been isolated from affected mango in Australia (Liew *et al*., 2016) [46]. All *Fusarium* species responsible for MMD cause similar disease symptoms.

Symptomology

Broadly three distinct types of symptoms were described by various workers. These are bunchy top of seedlings, vegetative malformation and floral malformation. Later, these were grouped under two broad categories i.e., vegetative and floral malformation (Varma, 1983a) [31].

Vegetative malformation

It is more commonly found on young seedlings (Nirvan, 1953) [53].

It is characterized by disrupting of apical growth resulting in several small flushes with quite short internodes at the apical ends of various branches. Symptoms of vegetative malformation include hypertrophied, tightly bunched young shoots, with swollen apical and lateral buds. Vegetative mango growth occurs in several intermittent flushes, separated by resting periods with no apparent growth (Davenport, 2009; Hernandez Delgado *et al*., 2011; Ramırez *et al*., 2014) [61, 62]. It may appear even in nursery stages on the main stem of the plants. These shoots bear small leafy structures appearing as if a crowded unhealthy and ugly looking mass. The multi-branching of shoot apex with scaly leaves is known as "Bunchy Top", also referred to as "Witche's Broom" (Bhatnagar and Beniwal, 1977; Kanwar and Nijjar, 1979) [67, 59]. The seedlings, which become malformed early, remain stunted and die while, those getting infected later resume normal growth above the malformed areas (Singh *et al*., 1961; Kumar and Beniwal, 1992a, Ploetz & Freeman, 2009; Chakrabarti, 2011; Freeman *et al*., 2014c) [48, 16, 5, 2, 5]. Vegetative malformation seriously affects seedlings and small plants in nurseries (Ploetz *et al*., 2002; Youssef *et al*., 2007) [49, 28].

Floral Malformation

It is the malformation of panicles and is more serious problem than vegetative malformation (Mahrous, 2004).

Floral malformation appeared in the panicles significantly impacts fruit production since affected inflorescences usually do not set fruit (Ploetz, 2001; Youssef *et al*., 2007; Ploetz & Freeman, 2009; Chakrabarti, 2011) [15, 28, 16, 2]. The primary, secondary and tertiary rachises become short, thickened and hypertrophied. Such panicles are greener and heavier with increased crowded branching. These panicles have numerous flowers that remain unopened and are predominantly male and rarely bisexual (Singh *et al*., 1961; Schlosser, 1971; Hiffny *et al*., 1978) [48]. The ovary of malformed bisexual flowers is exceptionally enlarged and non-functional with poor pollen viability (Mallik, 1963; Shawky *et al*., 1980). Both healthy and malformed flowers appear on the same panicle or on the same shoot. The severity of malformation may vary on the same shoot from light to medium or heavy malformation of panicles (Varma *et al*., 1969a) [31, 50, 51, 52]. The heavily malformed panicles are compact and overcrowded due to larger flowers. They continue to grow and remain as black masses of dry tissue during summer but some of them continue to grow till the next season. They bear flowers after fruit set has taken place in normal panicles (Singh *et al*., 1961; Varma *et al*., 1969b; Hiffny *et al*., 1978; Shawky *et al*., 1980) [31, 50, 51, 52] and contain brownish fluid (Prasad *et al*., 1965; Ram and Yadav, 1999) [48].

Malformed panicles may also produce dwarfed and distorted leaves (phyllody). There are various forms of malformed inflorescences, which include (Fig. 3): (i) a compact form with thick, green fleshy panicles that resemble cauliflowers (ii) a loose form with open larger-than-normal inflorescences, but with thick secondary branches (witches-broom) (Fig. 3B); and (iii) a combination of various compact and loose forms of vegetative and floral symptoms (Fig. 3C and D). Malformed panicles, which can persist on the tree until the following season as dry, black masses, serve as sources of inoculum for as long as they remain on the tree On the basis of compactness of panicles, malformed panicles are classified into different groups by different workers viz., heavy, medium and light (Varma *et al*.,1969c; Majumder and Sinha, 1972a) [31, 50, 51, 52]; compact malformed panicle, elongated malformed panicle and slight malformed panicle (Rajan, 1986) and small compact type and loose type (Kumar *et al*., 1993) [10]. The panicles of heavy type are very compact due to excessive crowding of flowers, keep growing to form large hanging masses of flowers, most of these dry up, and hang as brown discolored bunches, but some continue to grow till the next season. The medium types of malformed panicles are slightly less compact and persist on plant for a longer time than the normal panicles. The light type is only slightly more compact than the normal panicle and does not persist on the plant. Sometimes, a shoot tip may bear both types of panicles i.e., healthy as well as malformed. Less frequently, a healthy panicle may contain one or more malformed branches of a few malformed flowers or vice-versa. These partially infected panicles may bear fruits up to maturity (Kumar *et al*., 1993) [10]. In recent years, studies on dispersal patterns of conidia of *F. mangiferae* suggest aerial dispersal of inocula as the primary mechanism for fungal dissemination (Noriega-Cant_u *et al*., 1999; Youssef *et al*., 2007; Gamliel-Atinsky *et al*., 2009a, c) [28]. Gamliel- Atinsky *et al*. (2009c) [58, 57, 56] showed that infections are not systemic, with infections of apical meristems most probably originating and disseminating via conidia from malformed panicles. Malformed inflorescences and malformed vegetative growth serve as sources of inoculum from diseased panicles and malformed vegetative tissue, which disseminate passively in the air as conidia, or fall from dry, malformed inflorescences as dry debris (Gamliel-Atinsky *et al*., 2009b; Freeman *et al*., 2014a) [55]. After penetration, the pathogen colonizes the bud tissue but does not progress systemically into other plant tissues. Inflorescences from a colonized bud may emerge malformed, suggesting that a hormonal imbalance occurs in affected tissues and that an infection threshold is required for symptom development (Ploetz & Freeman, 2009) [16].

Management approaches

MMD remains mysterious as the different causative agents have been isolated throughout the globe. The management practices conducted over decades has been more or less effective under either laboratory or field condition. The most effective management strategies would include both control and the preventive measure of the disease.

1) Control approaches

The wide variability of microorganism being isolated from MMD increases the complexity for its control. The scientist has been very keen to tackle the mysterious malady through various methods i.e. physical alteration, chemical spray, plant growth regulators, antagonist concoction, and biocontrol agents. Some of the recent findings are targeted to decrease the incidence and increase the yield.

- Continuous removal of infected inflorescences over four-year results in the vast decline of disease incidence from 19.9% to 3.55% (Trial A) but the yield data doesn't vary significantly. The first two years of trial shows slight increment in yield which then remains constant. Later on an imidazole fungicide i.e. Prochloraz-Zn was sprayed at the interval of three weeks along with the removal of the malformed at regular intervals (Trial B) results in a notable decrease of incidence. The dual practice i.e. sanitation and Prochloraz spraying increases yield over three seasons by 26.9 t ha−1 (Magaritha H *et al*., 2018) [11].
- Among various fungicides, Prochloraz was the most efficient fungicide in inhibiting F. mangiferae *in vitro* requiring a 0.01 μg mL−1 concentration for inhibiting 50% fungal growth. The fungicide was found to be 90% protective and curative in greenhouse trials when the fungicide was applied continuously 14 days prior or post inoculation. (Freemana *et al*., 2014) [19].
- Almost all mangoes cultivars have been reported to be infected with the devastating malady i.e. malformation. The clipping of malformed branches at 45 cm distance followed by spraying of benomyl 50 WP spray at 2.0 gm L-1 water results in 70.37% reduction of disease incidence as compared to previous year (Zafar Iqbal1* *et al*., 2011) [29].
- Foliar application of nano-chitosan displays the increment in fruits yield as no of fruits and weight per plant increases and decrease in the incidence of malformation. (Osama Ahmed Zagzog *et al*., 2017) [13].
- Strong antifungal concoction was brewed from *Datura stramonium*, *Calotropis gigantea*, *Azadirachta indica* (neem) and cow manure (T 1) followed by methanol-water (70/30 v/v) extracts of *Datura stramonium*(T2), was found in trees sprayed with T(1) followed by T(2) at bud break stage and again at fruit set stage when compared with the control. Same concoction T (1) and T (2) were treated with *In vitro* culture of fresh malformed tissues in MS media showed no growth of any fungus in the media. However, the dried malformed leaves treated with T (1) and T (2) and *in vitro* culture done in MS media after foliar treatment appears the growth of *F. mangiferae* after the twenty-fifth days indicating that the concoction-brewed compost (T 1) or methanol-water (70/30 v/v) extracts (T 2) could not eliminate the pathogen but helped in controlling malformation by suppressing the activity of *F. mangiferae*. Mango trees sprayed with T 1 and T 2 revealed significant differences in percent fruit set and retention when compared with the control (K. Usha *et al*., 2009) [7].
- In a research conducted by Azz M. K. Azmy, The naturally MMD infected mango plants were treated with different Plant Growth Regulators. The application of Cultar (20 g a.i. /plant) results the 74.4% efficiency in reducing disease incidence and 83.7 kg of fruits / plant average yield which is the most effective growth regulator among other biocides and growth regulators followed by Agrotone (NAA), Berelex, Bio-Zeid, Blight Stop, AQ 10 and Bio-ARC being 71.2% efficiency and

78.3 kg fruits/plant,68.1% efficiency and 81.3 kg fruits/plant, 60.7% efficiency and 77.3 kg fruits/plant, 59.4% efficiency and 70.7 kg fruits/plant, 58.5% efficiency and 66.3 kg fruits/plant, 56.2% efficiency and 55.7 kg fruits/plant respectively. The most effective results were observed while the combination of cultar (Paclobutrazol) and Bio-Zeid (*Trichoderma album*) was treated against infected plant which gives 86.3% efficiency and 87.3 kg. Fruits/plant. (Azza M. K. Azmy. 2015) [1].

- Isolated *Streptomyces aureofaciens* was chosen as antagonists to *Fusarium moniliforme var. subglutinans*, the causative agent of MMD. Bioactive metabolites secreted by *S. aureofaciens* were measured as growth reductions of *F. moniliforme var. subglutinans*. The effectiveness of the bioactive metabolite produced by of *S. aureofaciens* at 1:5 concentrations against vegetative buds malformation disease of mango seedlings under artificial infested conditions were determined (Haggag *et al*. 2014) [6].
- An experiment was conducted in the northern province of South Africa where the Isolation of thirty-five fungal isolates was taken from infected orchards among them thirty-two were identified as the *Fusarium mangiferae*. Seventy-seven bacterial isolates obtained from the mango orchards and unrelated environments were screened for their antifungal properties. Among the all the isolated bacteria *Alcaligenes faecalis* were able to remarkably inhibit the pathogen growth (Veldman *et al*. 2017) [27].

2) Preventive approaches

Humans have been developing the practices of combating various malady suffered by their crops since the starting of the agriculture and the prevention approaches of mango malformation have been also based on those practices although the scientist has upgraded those principles of avoidance, exclusion, and resistance with several micro studies conducted over the century. Prevention approaches over some decade are more efficient due to the introduction of genetics, microbiology, biochemistry and other hidden science.

- The complete control of the mango malformation has remained ambiguous so the selecting of resistance varieties plays the vital role in reducing the disease incidence. The malformation was reported intensely in cv., Anwar Retail (56.63%). Mango cvs. Chaunsa and Malda were found to have the medium incidence of malformation i.e., 44.05 and 43.05%, respectively. Dusehri and Langra with values of 36.73 and 34.48% were marked to have low disease intensity. Among all four the cultivar the lowest malformation susceptibility was reported in Sensation i.e., 16.51% (Fayyaz *et al*., 2002) [4].
- Establishment of planting material for plantation should be done with pathogen-free nursery stock. Affected orchid should be avoided for the selection of scion material and the plants affected with malformation in the nursery should be removed and destroyed. Nurseries should also not be established in orchards, primarily where the incidence of malformation has ever been observed. These practices of avoiding the micro-organism in the planting material and medium have been commonly practiced in Egypt and India, which are the most severely affected areas (Ploetz, 2001) [15].
- Foliar Spray of 3% KNO3 over whole canopy promotes uniform flowering and spray of malathionat (1.5 ml a.i. /l) and chicken manures (2.5 kg/tree once a year) addition slower the epidemic development and lowers the level of initial and final disease ultimately lowering the disease progress curves as a result increase in fruits yield and the benefit-cost ratio (Thakur *et al*. 2000) [24].

Conclusion and prospect

The scientist has been continuously working to tackle the mysterious malady till today from the very first beginning of the disease incidence reported. Century-long researches were being conducted seeking the reason behind this malady and its control measures. In the long run of research work, different sciences like Microbiology, Biochemistry, and Genetics were being developed together and that has facilitated the researcher to dive into the depth of its causative agent, possible management practices and reduction in disease incidence. The most efficient method to manage MMD starts with the traditional principle of disease control i.e. Avoidance and resistance before the infection. When the disease infection occurs, the control measure must be carefully followed. The use of physical alteration followed by chemical spray, plant growth regulators, and antagonist concoction has been very efficient in controlling the disease incidence and ultimately increases the yield. Recent findings have concerned the scientist to be more focused on the bioactive metabolites secreted by different bacteria and researchers have been very keen to find the biological control agent against the mango malformation. Besides of all these achievements, malformation is still a severe threat to the mango growing farmers and the researcher need to focus on the analysis of the genetic diversity, antagonist feature of bacteria, gene expression of infected mango cultivars and development of disease-resistant cultivars. The genetically modified mangoes capable of resisting malformation could overcome the century-long worries of farmer across the world.

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