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METHODOLOGY FOR THE EVALUATION OF SYMPTOMS SEVERITY OF MANGO SUDDEN DEATH SYNDROME IN PAKISTAN

ASAD MASOOD[1], SHAFQAT SAEED[1], NAEEM IQBAL[1], MUHAMMAD TARIQ MALIK[2] AND MUNAWER RAZA KAZMI[3]

[1]University College of Agriculture,Bahauddin Zakariya University, Multan
[2]Mango Research Station, Shujabad, Multan, Pakistan
[3]Institute of Environmental Protection, National Agricultural Research Centre, Islamabad, Pakistan

Abstract

Mango (*Mangifera indica* L.) is an important fruit tree of the tropical and subtropical regions of the world. Presently, the mango orchards in Pakistan are badly infected with the destructive and latent disease, Mango Sudden Death Syndrome (MSDS). The apparent symptoms of this disease are gum oozing, bark splitting, rotting signs, vascular discoloration, canker formation and withered leaves attached over after dying of tree. Methodology for the assessment of mango sudden death in mango orchards was developed in devising a decision support system for timing management of disease. Disease incidence was observed on the collar portion including main roots of infected trees over total number of trees. The disease severity symptoms were recorded on the whole trees i.e., collar region, root zone, main stem, main limbs and leaves from four sides of tree. A disease scale was developed on the basis of visual observations of symptoms on infected mango tree. It comprised of seven scales ranging from 0= no disease sign, 1= 1-10% area of infection over total area observed, 2= 11-20%, 3= 21-30%, 4= 31-40%, 5= 41-50%, 6= 51-60% and 7= 61% to onwards regarded as maximum severity. According to the severity scale for assessment of MSDS, percent disease was calculated as mean value (totalling scale values of each disease symptom observed and dividing them by total number of parameters examined on mango plant) multiplied by 100/maximum scale value. On the basis of this disease assessment method, a sustainable management for mango sudden death syndrome would be devised.

Introduction

Mango (*Mangifera indica* L.) is an important fruit tree of the tropical and subtropical regions of the world. At present mango is grown over an area of 151.535 thousand hectares with production of 1637.947 thousand tones in Pakistan (Anon., 2007). There are numerous varieties of the mango like Sindhari, Doshari, Chounsa late, Summer Behist Chounsa, Anwer Ratol and Langra. Each having various fruit characters which determine the quality of the fruit and its market value (Jiskani, 2002).

The main reasonable abiotic constraints to low mango production and quality are water stress, extreme summer and winter temperatures, soil salinity, hard pan soils and nutritional deficiencies, particularly of Mn, Fe and Zn (Ploetz *et al*., 1996). Similarly, all the parts of plant, namely trunk, branch, twig, leaf, petiole, flower and fruit are attacked by a number of pathogens including fungi, bacteria that are already reported from Pakistan. Anthracnose, powdery mildew, bacterial blight, malformation and mango slow and quick decline mango diseases are well known (Khlaid et al. 2002; Kazmi *et al*., 2005). Besides all these, poor orchard management practices such as improper irrigation, intercropping, root injuries by deep ploughing and presence of infested plants are predisposing factors for disease development (Saeed *et al*., 2007).

*Corresponding author E-mail:bumblebeepak@gmail.com

Presently, the mango orchards are suffering from destructive and latent sudden death of mango in Punjab and Sindh Provinces of Pakistan. The attack of bark beetle, recently identified as *Hypocryphalus mangiferae* Stebbing, is also found on diseased mango tree(Masood et al. 2008) in mango growing countries of the world including Pakistan (Abbasi & Jiskani, 2006; Al Adawi *et al*., 2006). In Brazil, the same disease has been reported first time in 1960 (Batista, 1960) and in Oman during 1999 and affected up to 60% mango trees. The impact of mango sudden decline in USA, Brazil, Pakistan, Sicily and Iran on local mango cultivars was also reported (Jiskani, 2002).

In other mango producing countries, similar type of diseased symptoms like blight, canker, gummosis, twig blight, tip die-back and stem bleeding was also considered under the general term decline (Pernezny & Ploetz, 2000). Furthermore, Ploetz *et al*., (1996) has also observed defoliation, vascular discoloration, marginal necrosis and chlorosis of leaves and roots degradation. Al-Adawi *et al*., (2006) reported that sudden decline of mango in Oman is characterized by gummosis from the trunk, wilting, tree death, wood staining. These symptoms described above may be found alone or in combination of two or more symptoms in different mango orchards in Brazil or Pakistan (Ploetz *et al*., 1996; Iqbal *et al*., 2007). In Brazil, the development of Recife sickness and Seca diseases of mango like MSDS was also observed (Ribiero, 1980*)* from which *Ceratocystis fimbriata* was isolated from diseased tree (Iton, 1960). Van Wyk et al. (2007) reported that MSDS is primarily caused by *C. fimbriata* but typical symptoms of MSDS were also observed in mango plants after inoculation with *Lasiodiplodia theobromae* and this fungus was frequently isolated from declining mango trees and bark beetle in Pakistan (Saeed et al. 2008; Shahbaz et al. 2009).But, no single species of bark beetle was verified to be an effective vector for any fungal species (Masood et al.2008)

Therefore, it is highly important to assess the disease because infected plants serve as source of inoculums for the transmission of disease *via* soil, air and insect vector (Tariq *et al*., 2005). The disease assessment involves the measurement and quantification according to developed scale in order to predict the development of epidemics and in devising a decision support system for timing management. In other fruit crops like pear, disease incidence of pear brown spot disease was assessed by recording the number of infected leaves out of 10 leaves collected from 4 shoots per tree (Llorente *et al*., 2000). The severity of citrus greasy spot was measured and rated from 0 to 5 scales on basis of leaf area affected (Timmer *et al*., 2000). Estimation of sudden death syndrome of soybean was made on basis of symptoms (chlorotic, necrotic or defoliation) appeared on leaves in percent keeping in view the scale ranging from 0 to 9 (Luo *et al*., 2000). In field, severity in resistant and susceptible varieties of soybean was also assessed by using 0 to 10 scale corresponding to 0-100% tissue affected due the disease (Stetina *et al*., 2005). In Florida, severity of mango dieback symptoms was calculated according to allotted scale from 1 to 5 on diseased trees (Ramos *et al*., 1997). The severity of mango sudden death trees was observed on infected collar portion area out of total area. Keeping in view the scale, 0 means no sign of disease symptom, 1(1-25%), 2(26-50%), 3(51-75%) and 4(76-100%) vascular discoloration was recorded on infected collar portion of mango tree (Saeed *et al*., 2007). In this way, a numerical scale (0-5) was developed for blighted pome fruits representing as 1-10, 11-25, 26-50, 51-75 and 76-100% for the whole blighted twigs (Al-Dahmashi & Khlaif, 2004). MSDS is a very complex disease and different symptoms appeared on each portion of mango tree. The severity of MSDS on the whole mango tree i.e., collar, roots, stem, limb and leaves portions has not yet been evaluated. In this study, for the first time, a detailed methodology for the assessment of mango sudden death was developed according to the severity of symptoms.

Materials and Methods

A detailed methodology for MSDS was assessed as a percentage of disease incidence and as disease severity by randomly selecting 10 trees in each mango orchard. The study area was situated at a village "Faiz pur Bhuttia" near Khanewal Road, Multan (30.270^{o}N & 71.25^{o}E). The disease parameters were assessed by prescribed performa developed at University College of Agriculture, Bahauddin Zakariya University- Multan. The disease assessment parameters were critically examined according to the symptoms of MSDS in 5 portions of mango tree according to the allotted scale (0-7) which described later. The disease symptoms of MSDS on different parts of plant have been developed (Table 1).

Pictorial evaluation of MSDS symptoms on mango tree: The whole tree was divided into 5 different portions for evaluation of disease as shown in Fig. 1. Collar region is the underground potion of the stem up to the initiation of major roots which may be 6 inches or more than this length. That portion of tree was allotted the given severity (%) according to the scale (0-7), disease symptoms like oozing and canker formation as shown in Fig. 2, was observed as basic symptoms of MSDS on collar region.

The portion of major roots zone from collar region of stem was exposed up to 2 feet area all around the circumference of tree. The disease symptoms i.e., rotting, blackening and cankers were observed in the infected portion according to the scale as given in Fig. 3.

The main stem above ground was observed up to one meter length or less than this as the structure of each tree appeared. Stem portion all around the circumference was evaluated by recording MSDS symptoms (oozing, canker formation, bark splitting) according to the scale in Fig. 4.

The area of emerging points of main limbs started from the main stem was observed visually for the presence of disease symptoms. The symptoms of disease i.e., oozing, drying of twigs/branches, bark splitting were observed to summarize the infection according to the given scale as shown in Fig. 5.

The disease symptoms on leaves including curling/drying, severe shedding before the drying of the whole tree and attachment of leaves after the drying of the whole tree were also observed from the 4 sides of the tree according to the direction indicator i.e., east, west, north and south as given in Fig. 6. On the basis of symptoms and infected area of particular tree part was allotted scale from 0-7 showing 0-60% disease severity on each part of mango tree.

Disease evaluation parameters: The infection was identified on basis of symptoms i.e., cankers, oozing, rotting and blackening specifically on collar and root portions of mango tree. Therefore, disease incidence was calculated as number of infested plants showing above mentioned any single symptoms out of total numbers of mango plants observed.

$$\text{Disease incidence} = \frac{\text{Number of infected plants}}{\text{Total number of plants assessed}} \times 100$$

The disease severity on mango was determined according to the development of severity scale as the infected area of MSDS symptomatic portion of mango tree divided by total area and multiplied by 100 given below:

$$\text{Disease severity} = \frac{\text{Infected tissue area}}{\text{Total tissue area}} \times 100$$

Table 1. Description of mango sudden death syndrome (MSDS) symptoms.

Sr. No	Tree portion	Description of MSDS symptoms
1.	Collar region	i) Oozing of black/white material from infected area including gummosis
		ii) Canker formation (water sunken lesion/ corky appearance) with oozing of reddish brown substances from main stem/limbs which becomes black rust
		iii) Bad odour /filthy smell emitting from the infested area in final stage of decline
2.	Root Zone	i) Rotting of the main roots showing fungal infection
		ii) Blackening of roots zone area
		iii) Canker development
3.	Main Stem	i) Oozing of black/white material from infected area including gummosis and bleeding of wood sap.
		ii) Canker formation
		iii) Bark splitting/cracking: the longitudinal cracks/splits in the bark of main stem
4.	Main limbs/Trunk	i) Oozing of black/ white material from infected area
		ii) Drying of twigs and braches from top to downwards along with defoliation, blackening and shredding of outer bark of twigs/branches all around the main limbs of tree
		iii) Bark splitting/cracking
5.	Leaves appearance	i) Curling/drying of leaves due to stress all around the canopy of tree
		ii) Severe shedding of leaves before drying of whole plant.
		iii) Attachment of leaves after drying of whole plant. death of mango

By considering the mango sudden death symptoms, severity was recorded to evaluate the progress of disease on mango tree by observing each portion of tree. According to the development of scale on basis of disease severity, each infected portion corresponded to the scale in the following way: Scale-0= no sign, 1= oozing, cankers, rotting, blackening, bark splitting, drying of twigs or branches, curling or drying of leaves, shedding and attachment of leaves appeared on each portion i.e., collar, roots, main stem, main limbs and leaves of mango tree in range of 1-10 % severity, 2= 11-20 %, 3= 21-30 %, 4= 31-40 %, 5= 41-50 %, 6= 51-60% and 7= more than 60% area infected with mango sudden death symptoms.

Mean disease severity percent: According to original formula devised by McKinney, (1923) and reused by Cooke (1998), the mean disease severity percentage for each mango orchard was calculated with some modification keeping in view the allotted scale (0-7). In the same way, mean severity of MSDS (%) on individual tree was calculated by following formula as under:

$$\text{Mean severity (\%) on individual tree} = \frac{\text{Sum of numerical ratings on the whole tree}}{\text{Total number of disease symptoms on tree}} \times \frac{100}{7}$$

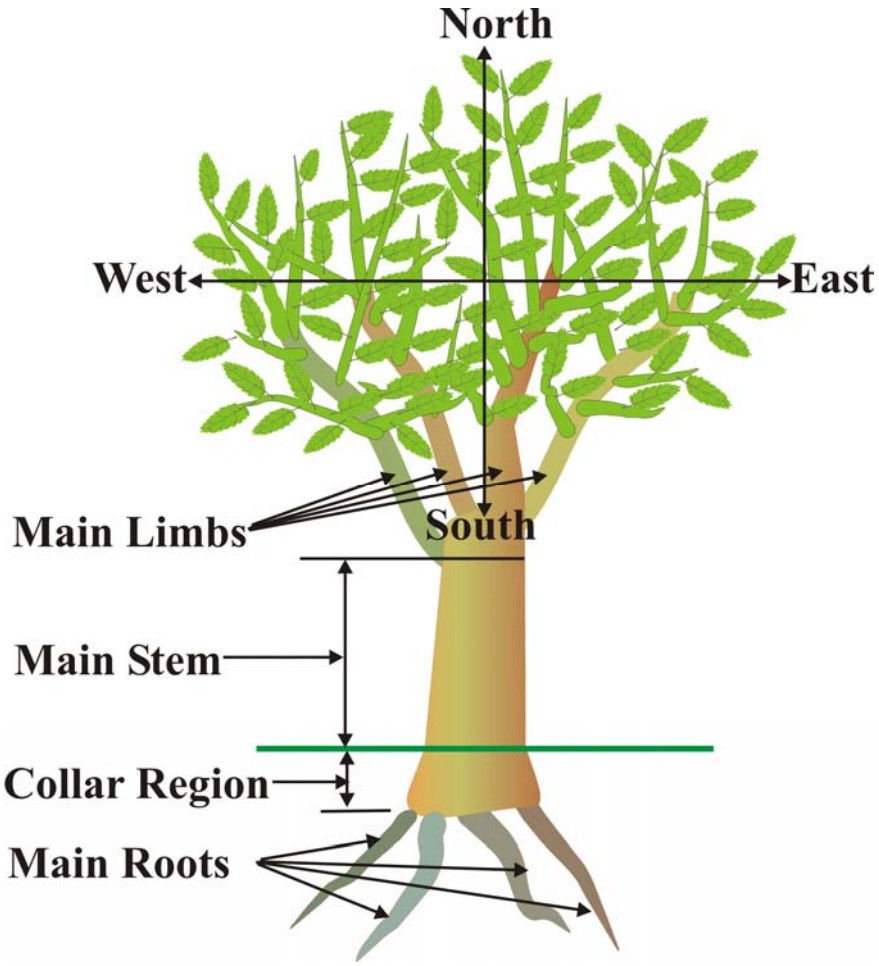

Fig. 1. Representation of different portion of mango tree for evaluation of disease symptoms.

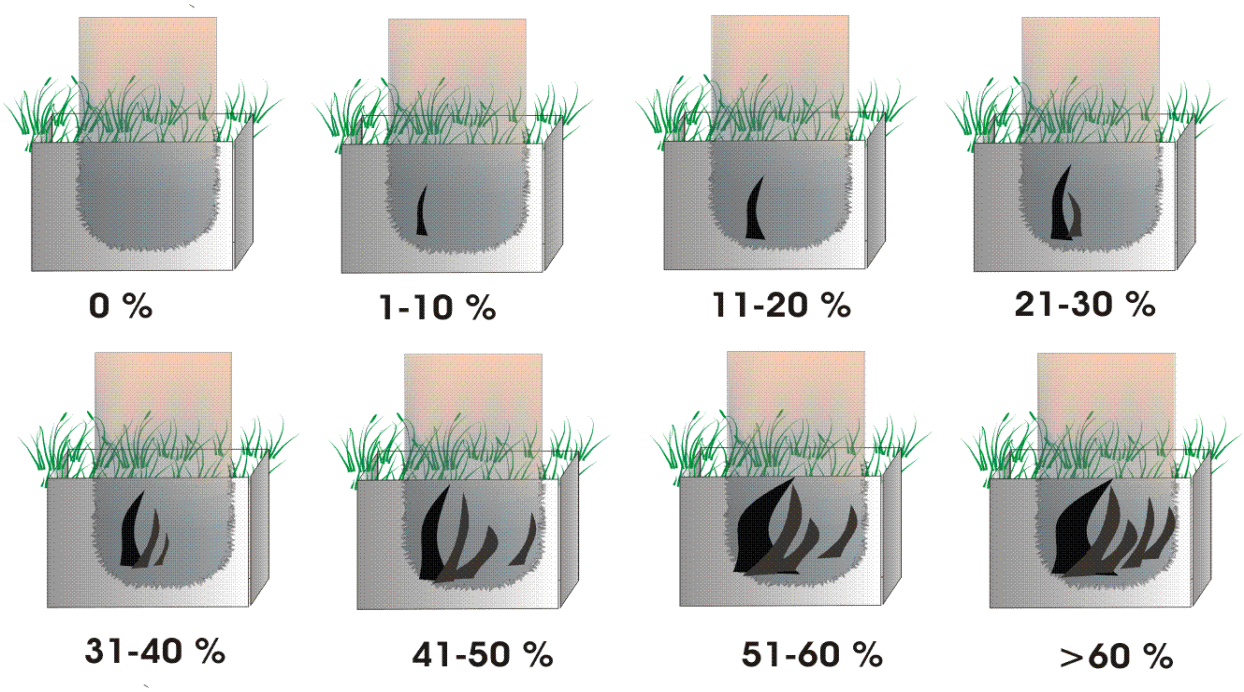

Fig. 2. Infection (%) on collar portion of mango tree according to scale (0-7).

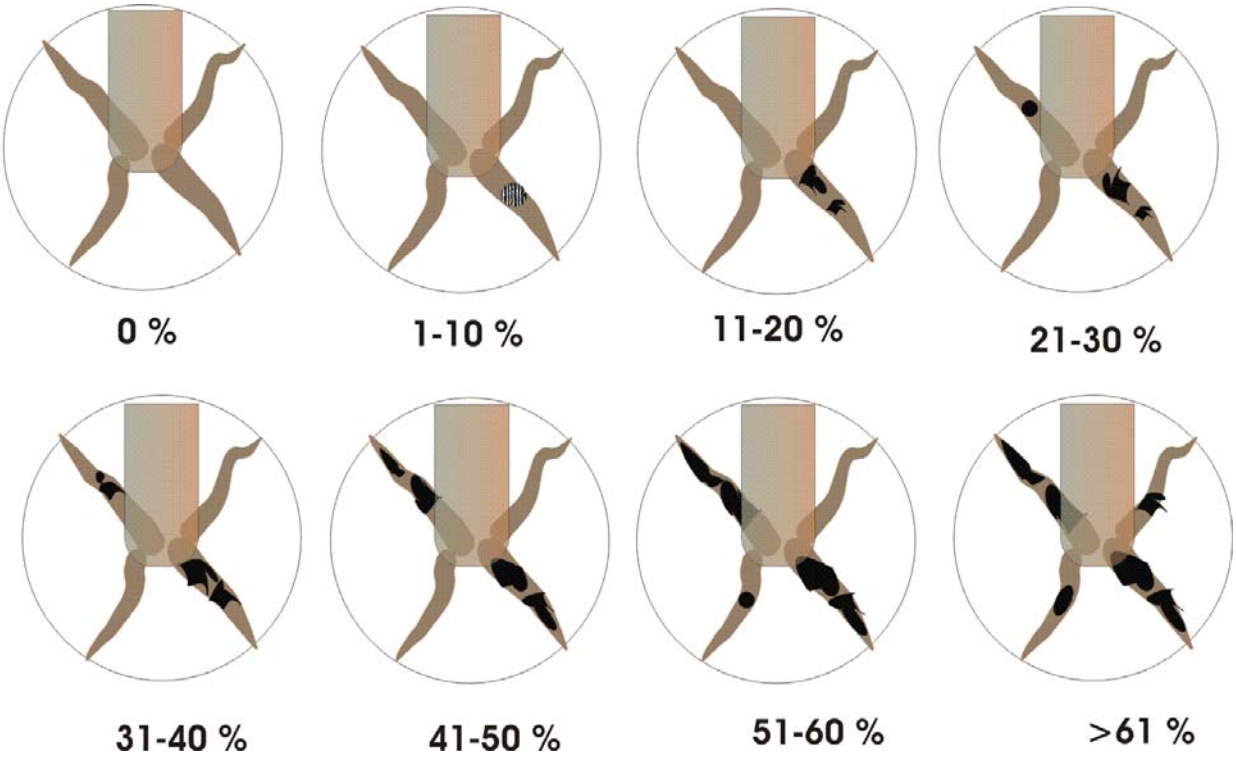

Fig. 3. Infection (%) on main roots of mango tree according to scale (0-7).

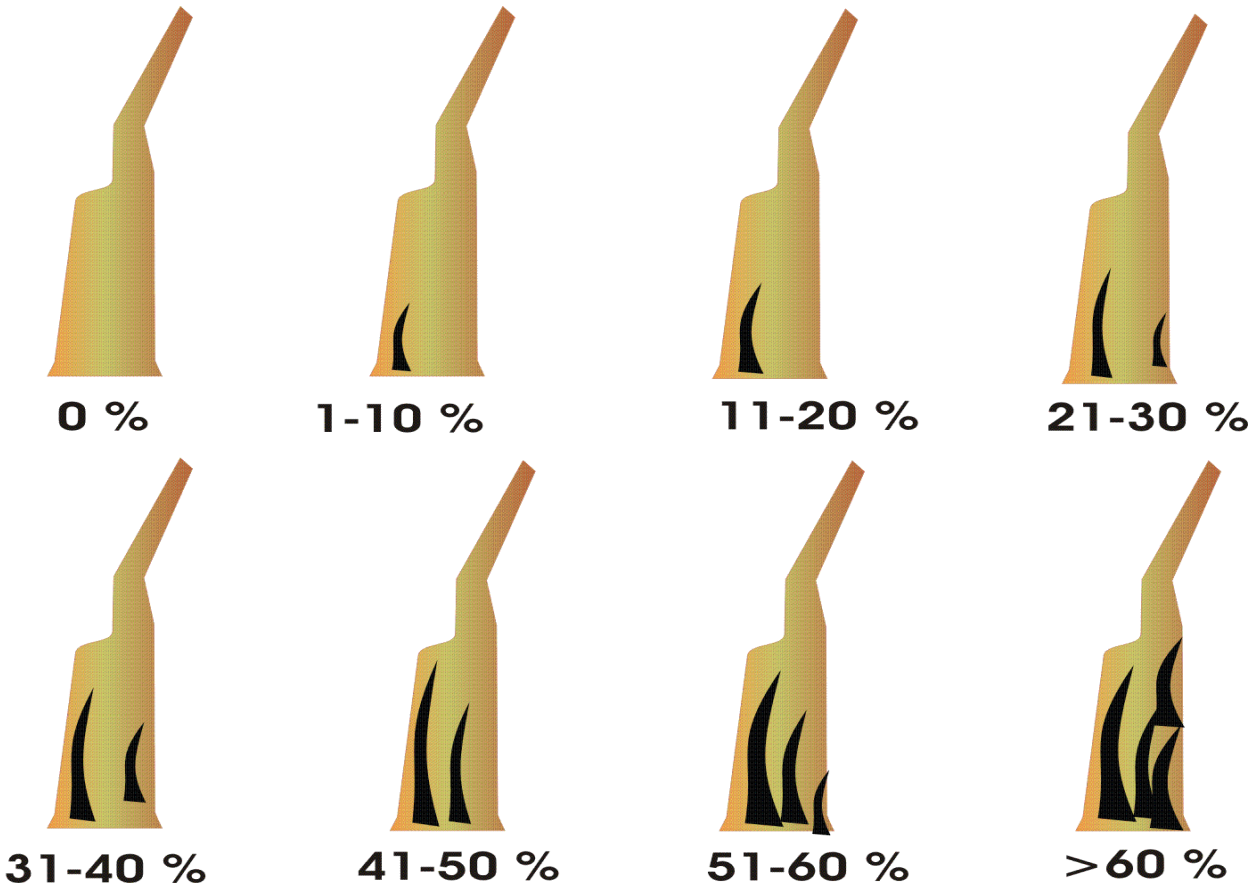

Fig. 4. Severity (%) on main stem of mango tree according to scale (0-7).

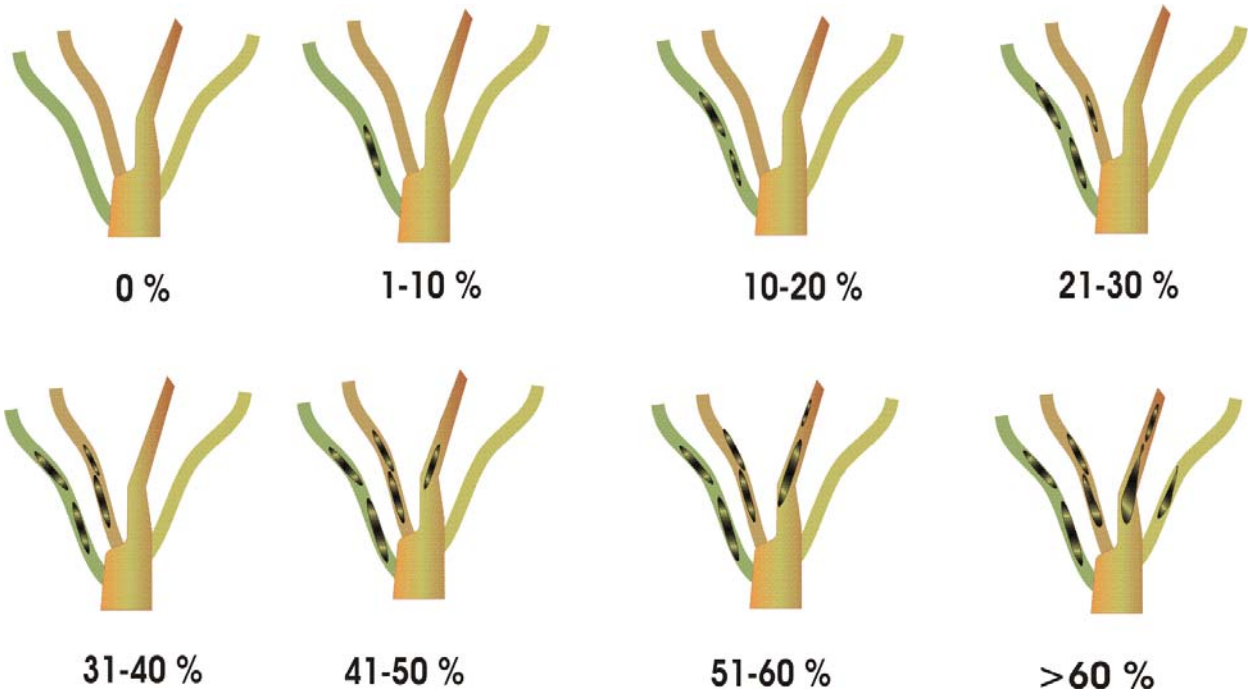

Fig. 5. Severity (%) on main limbs of mango tree according to scale (0-7).

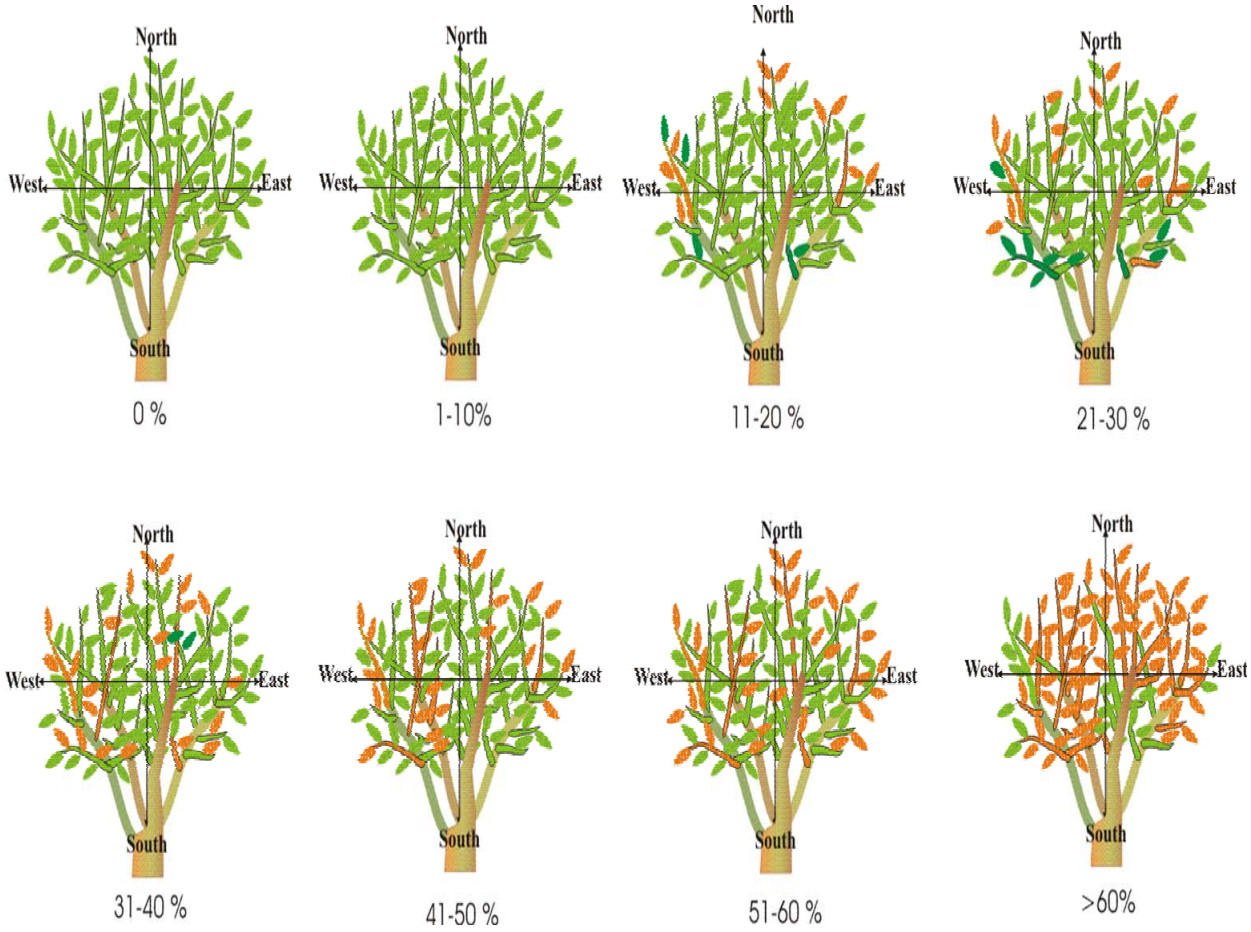

Fig. 6. Severity (%) on leaves from four directions of mango tree according to scale (0-7).

Results and Discussion

Disease symptoms of mango sudden death syndrome (MSDS): During the survey of orchards at least 10 trees per orchard were observed randomly for recording the mean disease severity percent with a method developed during research findings at University College of Agriculture B.Z University, Multan. All mango varieties grown in Pakistan have showed disease symptoms of MSDS. Initial disease symptoms were gummosis from the bark of the affected trees. These affected trees usually displayed other symptoms, including bark splitting, rotting sign on stem vascular discoloration beneath the gummosis. The leaves were withered and remain attached over after dying. When the infected portion was scraped, cankers became visible with oozing of black material and bad odour. Although, cankers on branches and stem were observed at initial and advance stage of disease but these symptoms lead to the progressive disease.

Evaluation of disease parameters for assessment of MSDS: The main symptoms of this disease were found as canker formation, rotting and oozing of blackish material on collar region and roots of mango tree. Therefore, MSDS incidence was focused at collar portion of mango tree while symptoms appeared on stem; limb and leaves were considered as leading to the progressive development of disease.

According to the disease assessment Proforma, data regarding MSDS symptoms on collar, roots, main stem, limbs and leaves were recorded from Fraz Farm, Khanewal area, Multan and calculated the mean severity (%) as described in Table 2.

Therefore, disease severity of different symptoms of MSDS like Oozing, cankers, rotting, blackening, bark splitting, drying of twigs or branches, curling or drying of leaves, shedding and attachment of leaves appeared on each portion i.e., collar, roots, main stem, main limbs and leaves of mango tree was described according to the allotted scale of disease from 0-7. The data regarding the average severity (%) on each mango tree portion was compiled which showed 4.28, 5.28, 24.28, 21.90 and 14.76% disease severity on collar, roots, main stem, main limbs and leaves of mango tree respectively. The overall disease severity of a tree was found as 14.09% and come under the disease severity scale-2 developed for the assessment of MSDS in mango orchard as given Table 2. Whereas, incidence of MSDS was calculated as numbers of infected trees (4) divided by total number of trees (10) observed during the orchard survey was 40% while keeping in view the infection symptoms only on collar and root portion of each tree.

The mean severity (%) for individual tree was calculated as 26, 14, 15, 8, 15, 9, 16, 13, 17 and 21 on each of the 10 randomly selected trees as shown the MSDS severity (%) of whole orchard (Table 3).

In this way, disease incidence percentage was assessed by measuring infections such as *Fusarium* wilt of tomato, wilt of banana, banana bunchy top and wilt of chickpea (Cohen *et al*., 2000). The standard diagrams illustrated the progress of disease on simple units i.e., roots, collar, leaves and on large composite units such as limbs. Such standard diagrams were derived from a series of disease symptoms in the form of line drawings according to developed disease scale (0-7) corresponding to 0 to > 60 % severity. In this way, disease severity is totally based on visual observation by allotting fixed scale using either descriptive of pictorial keys (Cooke, 1998). During this study, mean disease severity percentage was calculated by some modification according to MSDS symptoms keeping in view the severity scale (0-7) allotted to each infected portion of tree. Similarly, McKinney (1923) has devised a formula for calculating the mean infection and also used by Malik *et al*., (2005) with some improvement according to severity scale(0-4) for sudden death of mango in Pakistan. Therefore mean severity (%) was calculated as sum of numerical rating of all the MSDS symptoms appeared on the infected trees, divided by total number of mango trees observed during the survey and multiplied by 100/maximum disease scale 7.

Table 2. Mean and Average mango sudden death syndrome (MSDS) severity and symptoms appeared on single mango tree according to scale (0 to 7) at Multan.

Stage of tree	Symptoms	Mean severity (%)	Average severity (%)
Collar portion	Oozing	8.57	4.28
	Cankers	0.00	
	Rotting	2.85	
Root portion	Blackening	10.0	5.23
	Cankers	2.85	
	Oozing	10.0	
Stem portion	Bark splitting	62.85	24.28
	Cankers	0.00	
	Oozing	20.0	
Main limbs	Bark splitting	17.14	21.90
	Drying of branches	28.57	
	Curling/drying	44.28	
Leaf portion	Shedding	0.00	14.76
	Attachment of leaves	0.00	

Table 3. Mean mango sudden death syndrome (MSDS) severity (%) on 10 mango trees (cv. Chounsa) according to scale (0 to 7) in Multan region.

Tree portion	Symptoms	1	2	3	4	5	6	7	8	9	10
Collar portion	Oozing	1	0	1	0	2	0	0	0	0	2
	Cankers	0	0	0	0	0	0	0	0	0	0
	Rotting	1	0	1	0	0	0	0	0	0	0
Root portion	Blackening	2	0	1	0	2	0	0	0	0	2
	Cankers	2	0	0	0	0	0	0	0	0	0
	Oozing	2	2	0	0	0	0	0	1	0	0
Stem portion	Bark splitting	7	4	7	4	3	3	3	4	4	6
	Cankers	0	0	0	0	0	0	0	0	0	0
	Oozing	0	3	1	1	1	1	2	2	2	1
Main limbs	Bark splitting	2	0	0	1	0	1	2	1	4	1
	Drying of branches	2	2	3	1	2	1	2	1	2	4
	Curling/drying	4	2	1	2	4	2	4	4	4	4
Leaf portion	Shedding	0	0	0	0	0	0	0	0	0	0
	Attachment of leaves	0	0	0	0	0	0	0	0	0	0
Mean severity(%) on tree		**26**	**14**	**15**	**8**	**15**	**9**	**16**	**13**	**17**	**21**

It is concluded that the present detailed methodology evaluated disease severity symptoms on different portions of mango tree. On this basis, each portion of tree i.e., collar, root zone, stem, main limbs and leaves illustrated different disease symptoms according to severity scale of MSDS. With this methodology, disease incidence and severity was predicted on mango trees randomly selected from a single orchard like other fruit trees (Cooke, 1998). This assessment would be helpful in predicting the development of epidemics and in developing a decision support system for timely management before the collapse of mango trees in orchard.

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