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Spatial and Temporal Infestation of Mango Bark Beetle, *Hypocryphalus mangiferae* Stebbing (Coleoptera: Curculionidae) Found on Mango Sudden Death Trees in Orchards

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Abstract.- A preliminary survey of the mango orchards in Multan has been reported the coexistence of bark beetle attack on the trees infested with mango sudden death disease (MSDD). To extend this survey, the investigation of bark beetle in association with MSDD was planned in two Provinces of Pakistan *i.e.*, Punjab and Sindh. In each orchard, 10 trees were randomly selected and observed for the presence of disease symptoms *i.e.*, gummosis, cankers, rotting signs, vascular discoloration, bark splitting and wilted appearance. Whereas, damage of bark beetle was assessed by using 6 inches long stem section along the circumference on basis of number of holes at attack site. The infestation was higher in lower Punjab *i.e.,* Multan, Bahawalpur and Rahim Yar Khan as compared to upper Punjab *i.e*., Lahore and Kasuar while maximum infestation was recorded in Sindh Districts *i.e*., Tandojam Hyderabad, TandoAllahyar and TandoMuhammad. The assessment of *H. mangiferae* damage was also recorded on six mostly grown mango varieties *i.e*., Chounsa, Chounsa late, Ratol, Sindhari, Dosehari, Langra. The temporal damage of *H. mangiferae* in relation with disease was also recorded on cv. Chounsa round the year which showed that there was a strong relationship between disease severity and beetle infestation in terms of holes. Since the infestation of bark beetles was mostly observed in diseased mango trees, an integrated approach would be implemented for effective management in the orchards.

Key words: Mango, bark beetle, *Hypocryphalus mangiferae*, sudden death disease.

INTRODUCTION

Mango bark beetle, *Hypocryphalus mangiferae* Stebbing (Coleoptera: Curculionidae: Scolytinae) was formerly known as indigenous scolytid wood borer in mango trees (Mohyuddin and Mahmood, 1993), but now due to its role in disease transmission as a vector it has gained the status of primary pest for transmission of *Ceratocystis fimbriata* and *Lasiodiplodia theobromae*, the causal organisms of mango sudden death disease (MSDD) in southern Punjab, Pakistan (Masood *et al*., 2010a,b). Recently, Masood *et al*. (2010a) has also investigated the role of bark beetle on infected mango trees in host attraction which colonizes preferably on stressed trees (wounded, inoculated and disease symptomatic) in high number as compared with healthy tree and play significant role in disease epidemic. However, it requires feeding on twigs and branches of healthy trees for its complete development during its life cycle, otherwise its further development and breeding is stopped (Masood *et al.*, 2009). Likewise, in other insect-disease systems, elm bark beetles (*Scolytus scolytus, S. multistriatus*) were identified as effective vectors of *Ophiostoma ulmi and O. neo-ulmi* (Favaro and Battisti, 1993; Battisti *et al*,. 1994; Faccoli and Battisti, 1997). These beetles emerging from diseased trees are mostly contaminated with fungal spores and thus inoculate healthy host trees when feeding on the bark (Fransen and Buisman, 1935; Webber and Gibbs, 1989). The spread of MSDD, therefore, seems to follow similar pathways as Dutch elm disease in Central Europe (Lanier and Peacock, 1981**;** Brasier, 1987). Thus, it seems to be possible that MSDD and its rapid spread throughout the region during the last decade is the result of this new coincidence of bark beetle, an indigenous vector and an introduced pathogen.

Recently, incidence of MSDD was found 20% and more than 60% in Punjab and Sindh Provinces of Pakistan, respectively and 60 percent in Al Batinah region of Oman (Al-Adawi *et al.,* 2006; Kazmi *et al*., 2005; Saeed *et al*., 2006). This phenomenon had also been reported from some

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other parts of the world *i.e.*, Brazil and Oman (Ribiero, 1980; Al-Adwai *et al.*, 2006). The first initial visible symptoms of this disease include gummosis from the bark, bark splitting, streaking and vascular discoloration beneath the gummosis. The leaves of the trees wither out but remain attached with the dying tree (Masood *et al*., 2010b). The small pinhole of bark beetle and its irregular galleries were also observed on woody portion of diseased tree. During mango orchards survey in Multan District, Punjab, four different beetle species were collected from diseased tree and identified upto genera and one at species level (Table I). Among these, *Hypocryphalus mangiferae* was most frequently found in diseased mango trees (Masood *et al*., 2008). According to this preliminary survey, mango orchards situated within 20 kilometers were surveyed for disease and bark beetle (*H. mangiferae*) in all the four directions *viz.*, east, west, north and south. The orchards in eastern and western Multan had the maximum number of bark beetle attack as compared to northern and southern areas (Masood *et al*., 2008).

Due to increasing threat to mango industry, the survey area was extended to evaluate the incidence of the bark beetle and the disease in different mango production regions so that an area-wide management strategy could be devised for sustainable management of MSDD and its vector. Firstly, a survey of potential bark beetle species (*H. mangiferae*) was conducted in relation to MSDD in different mango growing areas of Punjab and Sindh provinces. Secondly, the assessment of *H. mangiferae* damage was also recorded on six mostly grown mango varieties *i.e.*, Chounsa, Chounsa late, Ratol, Sindhari, Dosehari, Langra. Thirdly, temporal infestation of bark beetle in relation to disease was investigated round the year in the mango orchard.

MATERIALS AND METHODS

Study area

In preliminary study, mango orchards were primarily surveyed for disease and bark beetle assessment in Multan (Punjab) comprised four directional areas *viz.*, towards Khanewal, Bahuaddin Zakariya University (BZU), Bund Bosan and Shujabad. This survey was extended to Southern Punjab (Lahore, Kasur), Northern Punjab (Rahim Yar Khan and Bahawalpur) and main mango growing areas of Sindh Province *i.e.*, Tandojam. Tando Allah Yar, Tando Muhammad and Hyderabad in 2007-08 (Table I). In each survey orchard, assessment of bark beetle and mango sudden death disease was conducted according to standard procedure (Masood *et al*., 2008). The bark beetle infested tree was identified on the presence of Pinholes, while diseased trees on the basis of characteristic symptoms of MSDD (Masood *et al.*, 2010b) *i.e.*, gummosis or oozing of material, wilting, bark splitting, curling/ drying etc. The most frequently sown commercial mango varieties *i.e.*, cvs Chounsa, late Chounsa, White Chounsa, Ratol, Dosehari, Sindhari, Langra and Fajiri were considered during the survey.

Table I.- Identification of beetles' species collected from diseased mango tree in Multan.

Common name[1]	Taxonomic ID	Location	Host condition and portion
Mango bark beetle	*Hypocryphalus mangiferae*[2] (Scolytidae:Coleoptera)	Faiz pur Bhuttia-Multan	Diseased, Healthy & branch, trunk
Ambrosia beetle	*Xyleborus affinis*[2] (Scolytidae:Coleoptera)	Faiz pur Bhuttia, Multan	Diseased tree & branch, trunk
Powder post beetle	*Sinoxylon* spp.[3] (Bostrichidae: Coleoptera)	Bosan Road, Multan	Dead and dry & Inside trunk
Nitidulid beetle	Nitidulidae[4] (Coleoptera)	Bosan Road, Multan	Dried tree & branch, trunk

[1:] Host plant, *Mangifera indica* L. and Collector Name: Asad Masood
[2:] MEFEIS stands for Museu de Entomologia da Faculdade de Engenharia de Ilha Solteira (Museum of Entomology of the Faculdade de Engenharia de Ilha Solteira), Ilha Solteira (city), state of São Paulo, Brazil (Carlos Flechtmann PhD), also help in ID by Dr.R.A.Beaver, 161/2 Mu 5, Soi Wat Pranon, T.Donkaew, A.Maerim, Chiangmai 50180, Thailand and Bjarte H. Jordal, PhD Department of Biology University of Bergen Allegt 41, NO-5007 Bergen, Norway.
[3:]Milos Knizek, Forestry and Game Management Research Institute Jiloviste – Strnady Praha 5 – Zbraslav CZ - 156 04 Czechia, Czech Republic
[4:] Not yet identified upto genus/species level

Estimation of bark beetle damage

The damage of bark beetle was assessed by using quadrates (15.24 cm) long stem section along the circumference at attack site designated as one sampling unit. Such three samples were drawn from base up to main stem section to estimate the damage of bark beetle in terms of number of holes as described by Lozano *et al*. (1997). Then, the average number of holes per tree was calculated for each variety tree.

Evaluation of disease symptoms

The disease severity was evaluated according to developed scale for mango sudden death on basis of presence/absence of the symptoms in mango orchard (Saeed *et al*., 2011). In order to define the severity of the disease, a simple scale was developed in the light of disease symptoms in different portion of tree *i.e*., twigs, branches, leaves and stem of individual plant from 1 to 5 corresponding to 0 to 100% disease severity previously developed by Ramos *et al*. (1991): 1) [0%] means plants free of disease; 2) [25%] early browning of leaf petioles and mid-veins and presence leaf necrosis in one or two branches; 3) [50%] presence of dried dead leaves remain attached, vascular browning, and evidence of pathogen invasion of vascular tissues by investigating the tree; 4) [75%] dried dead leaves and progressive defoliation appeared on many larger branches; 5) 100% progressive decline symptoms extended to major portions of the plant.

Spatial and temporal study of bark beetle

Keeping in view the damage of bark beetle in terms of holes and disease severity (%), total number of 90 trees was observed for each variety (cvs; Chounsa, late Chounsa, White Chounsa, Ratol, Dosehari, Sindhari, Langra and Fajiri) throughout the mango growing areas of Pakistan. To study the temporal variation of bark beetle damage in relation to disease, total number of 30 trees (cv. Chounsa) comprised of three bark beetle infested (source trees), 10 disease symptomatics (gummosis signs) and 17 healthy trees (without gummosis/ any sign) were continuously recorded on monthly basis from January to November, 2007. This specific orchard comprised 2000 trees with total area of 25 acres situated near Khanewal road, village Faizpur Bhattia (GPS position: 30.270º N; 71.250º E).

Statistical analysis

We used linear regression analysis to find the relationship between bark beetle damage in association with disease severity (%) on single cultivar round the year in one orchard and six cultivars observed during the survey. The data were analyzed using computer software XLSTAT (Anonymous, 2008).

RESULTS AND DISCUSSION

Infestation of bark beetle

During the survey, the community of bark beetles was composed of four beetle species, but *H. mangiferae* was regarded as the most frequently observed beetle in diseased or symptomatic trees as compared to other species (Table I). In the preliminary survey for disease and bark beetle, *H. mangiferae* assessment indicated that the bark beetle infestation was higher in lower Punjab *i.e*., Multan comprising of four directional areas, Bahawalpur and Rahim Yar Khan as compared to upper Punjab *i.e*., Lahore and Kasur. The maximum infestation was recorded in Sindh province *i.e*., Tandojam, Hyderabad, Tando Allahyar and Tando Muhammad (Table II). Similarly, Saeed *et al*. (2006) reported that sudden death of mango is formally spreading in large areas in two provinces, in the Punjab and recently in Sindh. The attack of this disease in the province of Punjab started in 1995 from the district Muzafargarh and now it prevails in every orchard of the mango (Kazmi *et al*., 2005). Initially, different orchards of mangoes were surveyed in Multan region and 20 percent of the trees had died due to this disease and different bark beetle species were also collected from sudden death mango trees (Masood *et al*., 2008; Saeed *et al.,* 2009). Among four different beetle species, *H. mangiferae* was found frequently with MSDD trees (Masood *et al*., 2008). Besides Pakistan, Al-Adawi *et al*. (2003) reported that 60% of the trees of mango were infected in the parts of Al Batinah region of Oman. Likewise, Dutch elm disease (caused by *Ceratocystis ulmi*) has recently been reported to spread in Europe, Central Asia and North America

Table II.- Spatial bark beetle infestation in relation to mango sudden death disease in Punjab and Sindh Provinces of Pakistan.

Sr. No.	Orchard location	GPS location of orchard	Number of orchards	Total trees	Sum disease trees	Diseased trees (%)	Sum beetle trees	Beetle trees (%)
Multan Region	Khanewal Road	30.270ºN 71.250ºE	18	180	27	15	16	8.88
	BZ University	30.263ºN 71.506ºE	17	170	24	14.11	14	8.23
	Bund Bosan	30.266ºN 71.494ºE	14	140	32	22.85	9	6.48
	Shujabad	30.255ºN 71.513ºE	16	160	37	23.12	8	6.66
Southern and Northern Punjab	Lahore	31.410ºN 73.050ºE	4	40	2	5	2	5
	Kasur	30.650ºN 73.120ºE	2	20	1	3.33	1	3.3
	Rahim Yar Khan	28.390ºN 70.320ºE	5	50	5	10	4	8
	Bahawal pur	29.400ºN 71.660ºE	3	30	6	20	2	6.66
Sindh Province	Tandojam	25.430ºN 68.540ºE	2	20	4	20	5	25
	Tando Allah Yar	25.410ºN 68.670ºE	4	40	6	15	5	12.5
	Tando Muhammad	25.460ºN 68.720ºE	2	20	3	15	2	10
	Hyderabad	25.370ºN 68.350ºE	3	30	2	6.66	4	13.33

by various elm bark beetles of genus *Scolytus* (Lanier and Peacock, 1981; Brasier, 1987). The bark beetles, *Scolytus scolytus, S. multistriatus, S. kirschi* and *S. laevis* are involved as potential vectors in the dissemination of the disease (Lekander *et al*., 1977). MSDD is also reported from Oman to be consistently associated with a bark beetle, *H. mangiferae* possibly acting as a wounding agent (Al Adawi *et al.*, 2006). In Brazil, *H. mangiferae* is also reported as the potential species responsible for disseminating *Ceratocystis fimbriata* in the development of Seca diseases of mango (Ribeiro, 1980*)*. The other beetle species, *Sinoxylon conigerum* also attacks on mango tree (*Mangifera indica* L.) in Brazil (Filhoi *et al.*, 2006). It is suggested that as *H. mangifera* and *S. cognigerum* has already been reported from Oman and Brazil, respectively, in mango orchards. These two species are also reported from the present study from mango orchards of Pakistan. Besides *H. mangifera* and *S. cognigerum*, ambrosia beetles, *Xyloborus* spp. mostly prefer to attack stressed, dead, dying woody plants and wounds or dead area in living trees (Masood *et al.*, 2008). Baker (1972) also observed the same preference in anoher species of this genus but ambrosia beetles of the tribe Xyleborini feed on fungi that are inoculated into galleries by the adult females. A vascular wilt ambrosia fungus (*Ceratocystis* spp.) has been recovered from infested mango which is believed to be the fungus on which the beetle feeds (Rabaglia, 2005).

Severity of bark beetle damage

The assessment of *H. mangiferae* damage on basis of number of holes per unit bark area was also recorded on six mango varieties *i.e.*, Chounsa, Chounsa late, Ratol, Sindhari, Dosehari, Langra. No variety was found resistant against the attack of bark beetle and showed differential damage in terms of holes. The maximum damage was observed on Sindhari (320±381 holes) followed by Chounsa (123±182 holes) and Dosehari (102±48 holes). Whereas, the minimum number of holes was found on Langra (41±24) and Ratol (48±36) and showed comparative resistance against the attack of bark beetle. The disease severity was more or less the same on all varieties except cv. Langra which showed minimum infestation of sudden death disease (Fig. 1). The survey results would indicate a baseline data regarding the infestation of disease and bark beetle which could also be helpful in developing a resistant/tolerant variety in future breeding program. On the whole, there was positive relationship between number of holes made by bark beetle and disease severity recorded on six mango varieties (Fig. 2). The temporal variation in damage of *H. mangiferae* in relation to disease was also recorded on a single variety of cv. Chounsa round the year which showed that there was a strong relationship between disease severity and beetle infestation in terms of number of holes (Fig. 3). The high level of disease severity was observed on source tree and disease symptomatic tree in comparison with healthy trees. Similarly, the trend of bark beetle dispersal has increased significantly from May to August and then tended to decrease from October to November on diseased and symptomatic trees. Healthy trees on the other hand showed the least preference of bark beetle compared with diseased and symptomatic trees (Fig. 4). The trend line of infestation indicated that maximum attraction of bark beetle was found on diseased trees (source tree suitable for beetle breeding) and minimum towards healthy trees (only require maturation feeding before going to breed in infested trees). The dispersal activity of *H. mangiferae* was also observed in an orchard of Multan region (Punjab) which indicated its maximum activity during May and decreased in the months of June and July due to rains then its population increased again in September (Masood *et al*., 2009; Saeed *et al*., 2010), which was also in accordance to findings of the survey. Therefore, activity period of mango bark beetle should be considered while devising management strategy.

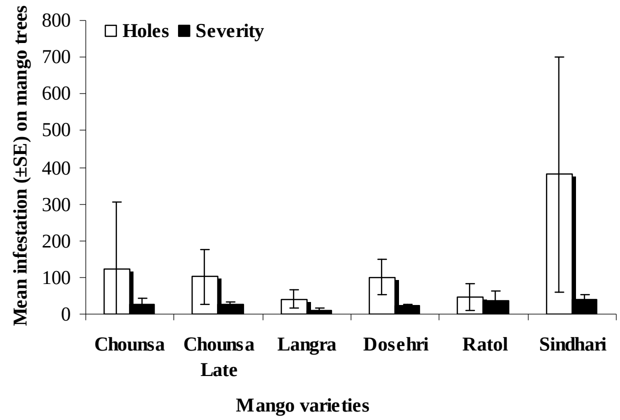


Fig. 1. Estimation of damage of mango bark beetle in relation to mango sudden death disease observed on six cultivars (N=90).

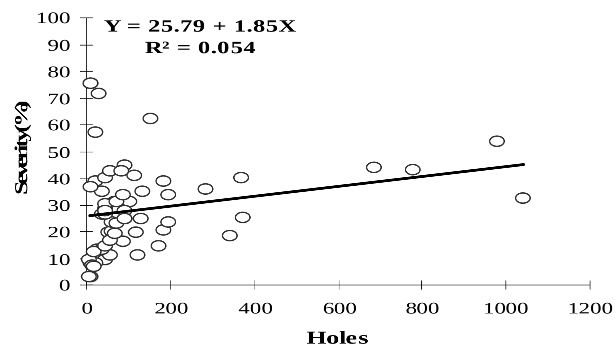


Fig. 2: Relationship of damage pattern of beetle and disease severity on six cultivars.

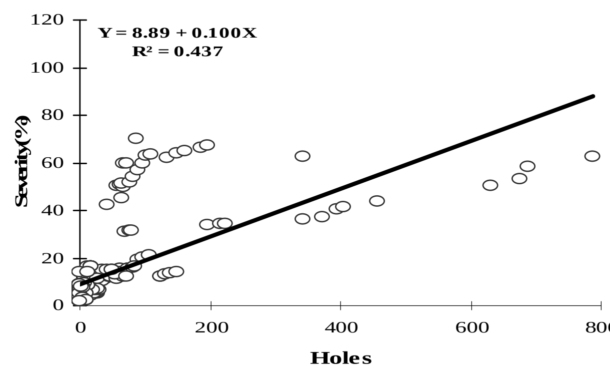
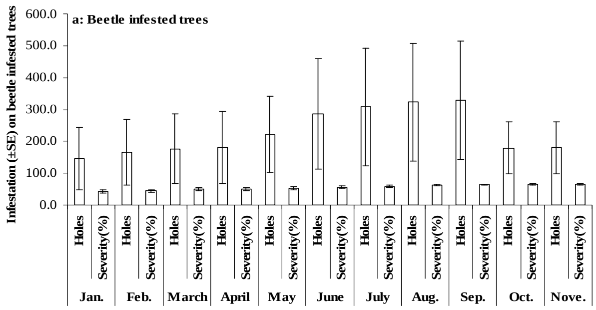
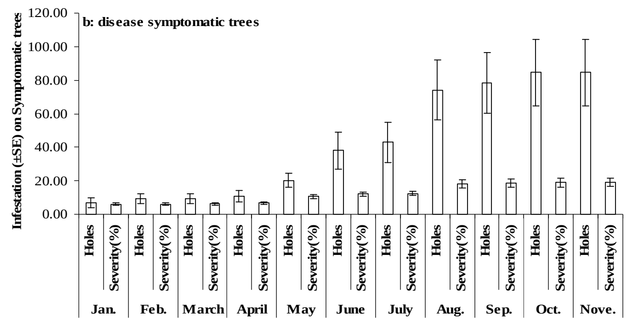


Fig. 3: Relationship of damage pattern of beetle and disease severity on single Cv. Chousa round the year.

Most species of wood-tunneling bark beetles are considered as opportunists that attack stressed or



A



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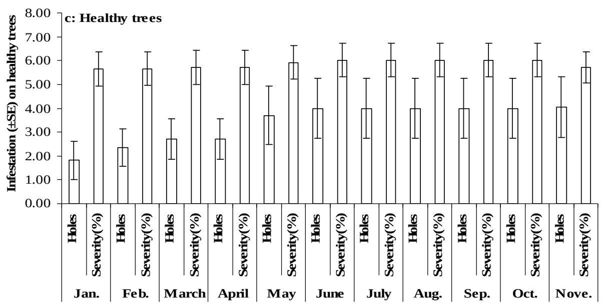

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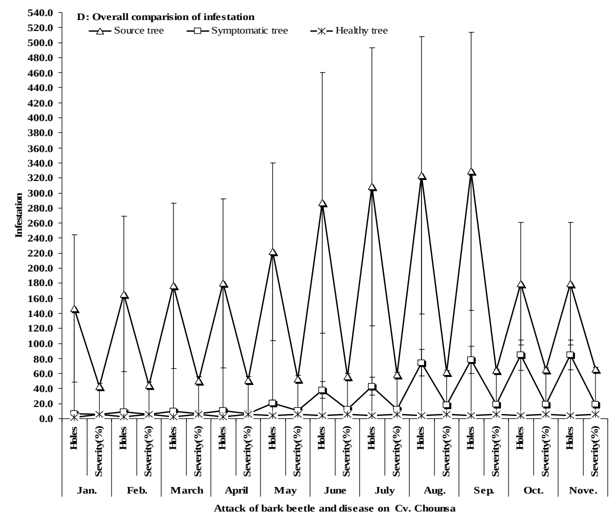

D

Fig. 4. Mean infestation (±SE) of beetle‘s attack and disease severity (%) on Cv. Chounsa; A, beetle infested; B, disease symptomatic; C, healthy trees; D, overall comparison of infestation among A, B and C.

diseased trees and prefer to colonize only in recently cut, wounded, or weakened portions of woody plants (Wood, 1982). Besides, its consistent association with sudden death disease, the attraction of bark beetle in terms of holes was assessed at different decline stages of mango plants *i.e.*, symptomatic, disease inoculated, stressed (due to salts), wounded while healthy and dried plants served as control in lathe house conditions. The cumulative results showed that inoculated and symptomatic plants were preferably attacked by beetle which is also observed in this study (Masood *et al.*, 2010a). During the life cycle of *H. mangiferae* it may also require maturation feeding on fresh healthy mango trees or branches before colonizing on diseased tree that is why beetle holes were also found on healthy tree (Masood *et al.*, 2009). Likewise, the disease cycle of Dutch elm disease starts when a new generation of *Scolytus* emerges from diseased elm bark of tree. The beetles loaded with fungal spores, seek out bark for fresh breeding material often feed in twig crotches of healthy elm (Choudhury, 1979). It is the means by which *Ceratocystis ulmi* infects healthy elms, the wounds made by beetle serving as infection courts for the pathogens (Webber and Gibbs, 1989**)**. An infected tree may succumb to the disease within a single season or even several years but eventually the bark of declining elm can be colonized by breeding scolytids. This period of bark colonization by insect-fungus usually occurs during winter, although it can be compressed into a few weeks of summer and ensures that pathogen and vector are reunited by beetle emergence in early summer. Since, the elm bark beetles have no specialized organs for the fungal transport and eventually exposed to extreme environmental fluctuations during beetle flight. Besides environmental conditions, numbers of viable spores are also important for progressive infection (Webber and

Gibbs, 1989). Fraedrich *et al*. (2008) suspected that the fungal pathogens first infect healthy trees during tunneling process followed by the attack of the beetle, *Xyleborus glabratus* on redbay in coastal plain counties of Georgia and southeastern South Carolina. Thus, bark beetles serve as a facilitating agent for the development of fungi within the whole tree and the trees later become susceptible for colonization of beetle. That is why, laurel wilt pathogen may be inoculated into stems and branches of healthy trees by tunneling of beetle in a manner similar to the transmission of the Dutch elm disease fungi followed by bark beetle vectors during maturation feeding process in elm trees (Webber and Gibbs, 1989; Jacobi *et al*., 2007). It is reported in mango orchard that wounding holes made by bark beetle can be served as penetration points for the spores of fungi and thus infection may be transmitted by the mango bark beetle (Al Adwai *et al*., 2006; Masood *et al*., 2012b).

Therefore, spatial and temporal infestation of bark beetle in relation to disease would provide baseline data for developing an integrated management strategy for the disease as well as its vector. Whereas, infestation of bark beetle and disease on different mango varieties (Fig. 1) would provide valuable information for development of resistant/tolerant cultivars in breeding program as future prospective. As many of the species are found simultaneously in the infested mango tree, only bark beetle species which was more frequently recorded on mango tree during the survey, *H. mangiferae* (Coleoptera: Scolytidae) was identified and the other species of beetles, Powder post beetle, *Sinoxylon* sp. (Bostrichidae), Nitidulid beetle (Nitidulidae) and Ambrosia beetle, *Xyleborus* sp. (Scolytidae) were identified up to family and generic level (Masood *et al*., 2008). Based on disease transmission through bark beetle and attraction study of bark beetle towards stressed as well as healthy plants, it is concluded that *H. mangiferae* was established as a potential vector of MSDD in Pakistan (Masood *et al*., 2010a,b). Therefore, the management of *H. mangiferae* was developed by using different insecticides *i.e*., imidacloprid, emamectin benzoate, deltamethrin, bifenthrin and chlorprifos. The efficacy of insecticides was classified on basis of number of emerged adult beetle while comparing with control (to which no insecticide was applied) which resulted in descending order: Emamectin benzoate < imidacloprid < chlorpyrifos < deltamethrin < bifenthrin (Saeed *et al*., 2011a). Therefore, integrated disease management practices would be requested through which mango sudden death disease can be minimized by following the best management practices *i.e*., field sanitation, ring formation around tree trunk, proper pruning, balanced use of macro and micronutrients, avoid deep ploughing and intercropping and plant protection measures according to the situation of pest and disease (Malik *et al.*, 2004; Saeed *et al*., 2009; Masood and Saeed, 2012). Therefore, integrated crop management (ICP) practices should be implemented on large scale to increase sustainable yield and quality of mangoes in Pakistan for ultimate increase in foreign exchange earnings as future prospective.

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