Dying of flora: A morphological study

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Abstract: This study is based on the author's experience and it is established that the real cause of flora's dying and drying are non-traditional pathogens namely ENOs, which are non-contagious like those for AIDS, affect exposed xylem tissues, remain therein, and lead to a GRENE Syndrome for which no treatment is discovered as yet. It is a challenge for the biological scientists to analyze the ENO affected plants from the point views of anatomy, physiology, bio-chemistry and many other issues mentioned in this study. The issue merits special consideration in view of the fact that the commercial, ornamental, timber, and fruit plants are being swallowed.

Keywords: GRENE Syndrome, Flora, ENOs, Morphological Features

INTRODUCTION

In earlier studies on the subject [Ellahi (2005) and (2006)], some elementary issues about the guided, restricted and extra-natural environmental (GRENE) Syndrome were discussed, which are operated by the extra-natural organisms (ENO). Therefore, duplications can not be avoided. Further, additional information based on empirical studies conducted by the plant pathologists was provided in the ‘Third Seminar on Shisham Dieback held at the Punjab Forestry Research Institute (PFRI) Faisalabad’ and merits to be incorporated in this study.

In addition to the biological issues, an attempt is made to discuss economic implications of the GRENE Syndrome under varying agro-climatic conditions. In this respect, a salient example relates to declining yields for apples, apricots, plums, peaches, grapes, etc. since 1998-1999 in Balochistan valleys, while mango blight in Punjab was conspicuously observed in 2005-2006 and thereafter.

The economic implications of the GRENE Syndrome and Methods and materials used for a study are discussed hereafter. These are followed by a review of literature describing main features of flora's dieing and drying. Thereafter, additional morphological features noted by the author are discussed. Finally, basics of the main issue and results are highlighted, which are followed by the recommendations to avoid the indicated problems.

Economic implications

The mango blight, caused by an unknown disease, has reportedly claimed a heavy toll in 2005-2006 in the wake of relatively lower temperature as compared with the average for the previous 6 to 7 years. This observation is not unprecedented as per acre fruit yield in hilly Balochistan for apples, apricots, plums, peaches, grapes, etc. has been declining at 6 to 17 per cent per annum compound during 1998-1999 to 2003-2004 [Ellahi (2007)]. An early on-set of decline in fruit yields in Balochistan (having cold climatic conditions as compared with plains) viz-a-viz Punjab seems to exhibit a negative correlation between GRENE Syndrome and temperature. An empirical analysis was carried out to see growth or otherwise in area, yield and production of salient fruits during 1997-1998 to 2003-2004. The said data were obtained from the Agricultural Statistics of Pakistan [Government of Pakistan (2009)]. Empirical results are provided in Table 1 and depicted in Figure 1, which show that change in yield and production for citrus and mango until 2003-04 has been nominal. On the other hand, a tremendous decline in yield of cold region fruits was experienced, where the highest toll was observed for apples, which is the most commonly used item. It may be noted that most of these fruits are budded/grafted or pruned extensively and amenable to attack of GRENE Syndrome.
Methods and materials

This study is mainly focused on observing morphological symptoms of the flora subject to the dieing and drying process. Thus, it is based on a review of existing literature on the subject and a purposive selection of flora and fauna involved, directly or indirectly, in the scenario being researched into. The Lahore city was taken as universe and flora were selected in a way that maximum variation in morphological features may be captured.

Morphological features reviewed

The Eucalyptus (*Eucalyptus globulus*) die-back was noted and widely discussed in Australia during late 1970s and early 1980s, which contradicts with Gul’s (2004) recommendation of growing it on account of being resistant to die-back. It is generally claimed that infection enters into a plant from injured tips of the branches, which start dieing-back until the entire plant does so (Khan, 2006). Khan (2004) opined that dieing-back is not merely a disease, but a syndrome with multiple causes and symptoms. Jamil (2004) reported that Australian scientists are still working on dieback and root rot diseases, which are claimed to be caused by *Phytophthora cinnamomi* and effectively suppressed by using composted farm yard manure (FYM) from chicken origin. The use of FYM was also recommended in a meeting of Task Force chaired by Mr. M. Shafi Niaz on June 2, 2001 at the PFRI Faisalabad.

In Pakistan and other countries of the sub-continent, such as India, Bangladesh, Bhutan and Nepal as well, the disease came to surface with a large scale appearance of partial or complete drying of *Shisham* (*Dalbergia sissoo*) in late 1990s. The start of problem, claimed to be caused by fungi (*Ganoderma lucidum* and *Fusarium* species) is reportedly dating back to the late nineteenth century in a sporadic way. Afzal (2004) confirmed the existence of *Ganoderma lucidum* as main causal organism, but the University of Agriculture Faisalabad viewed that Pin Hole Borer and Horn Beetle were causing the problem (PFRI 2004).

Chaudhry and Ahmad (2004) reported spreading of the said disease to Kikar (*Acasia nilotica*), Semal (*Salmalia melabericum*), Mango (*Mangifera indica*), Citrus species, etc. growing on farm-lands. Further, the disease pattern is shown to be atypical, such as vertical tree division with one each green and dry wood, thinning of crown, leaves turning
yellow or tan coloured, cankers on southern side of trees, exudates of black or red colour, insects attaching main stem and many plants turning completely dead and dried [Chaudhry and Ahmad (2004)]. This is reportedly observed among a wide variety of trees in all ages on the campuses of a large number of educational institutions in Peshawar. Disease symptoms reported by Gill (2004) are quite similar to those brought out by Chaudhry and Ahmad (2004). However, additional symptoms included withering of stem/branches upward from infection point, disease moving down with roots and turns, bark splitting and drying followed by infection in roots and collapse of entire plant. Finally, Keerio (2004 and 2006) reported disease incidence to be as low as about four per cent in Sindh, where two third of it related to Shisham which is consistent with an overall concern.

Ellahi (2005 and 2006) reported that the unidentified pathogens, namely ENOs, stay in xylem tissues of the flora, survive on raw food, which is soluble, and cause the instant disease. The Secretary to Government of Punjab for Forestry, Fisheries and Wildlife, in his Keynote address to the Third Seminar on Shisham Die-back, held at the PFRI, Faisalabad, on May 11, 2006, related the disease to the xylem tissues of plants and ascribed fungal attack as a secondary cause. The location of xylem tissues in plant’s vascular column for root and stem is shown in Figure 2.

As a part of research efforts, two major studies were undertaken by the University of Punjab (Bajwa and Mukhtar 2006) and the Ayub Agriculture Research Institute (AARI), Faisalabad [(Idrees et al. (2006a) and (2006b)]. The University of Punjab concluded that Fusarium solani was the main cause of the disease, while the AARI found Botryodiplodia theobromae as the main causal organism [Idrees et al. (2006a)]. Both the said studies collected large number of samples of Shisham seed, bark, roots, soil etc. from a wide range of Shisham growing districts of Punjab, but none of them included xylem tissues in their analytical process. The culture medium used was starch, dextrose and agar, which is contrary to the natural requirement of the xylem infection. Thus, despite similarity in methodological procedures, the said two studies led to divergent results, which is a severe question mark on their credibility and efficacy from the curative points of view. This has two implications, first; the identified fungi is a secondary cause and second; the primary cause (ENO) stay in xylem tissues and can not be identified from the dried and dead wood.

Additional morphological features

Apart from the morphological features reviewed above, there are additional ones which merit consideration. As seen from Photo 1, these include, stem and roots getting zigzag [Shisham, Gulchin (Plumiera obtusa) and Amaltas (Cassia fistula) in Photos 1-1, 1-2, 1-14 and 1-15], branches saddle-shaped and atypical [Shisham in Photo 1-3 and guava (Guava juajava) in Photo 1-4], twisting independently [Sarv (Cupressus torulosa) and Eucalyptus in Photos 1-5 and 1-6] and around each other (Photo 1-7) and wood separation from living part as seen from Photo 1-8 for Jamun [Syzygium jambos (linn) specie cumini].

Selected ENOs and their actions, mainly for Semal and Amaltas, may be seen from Photos 1-9 to 1-20 of Photo 1. In case of Semal, Photos 1-9 shows ENOs’ diversion at a branch out point into another branch, which may not be visible in some cases but the existence of ENOs therein can not be ruled out. Four pieces of a branch from Semal (the largest starting from extreme right to the smallest at the extreme left) are in Photo 1-10 and their remaining three parts towards apex are in Photo 1-12. The first two parts from the extreme right in Photo 1-10 hardly show any signs of infection, but the remaining two exhibit traces only. This effect is seen more clearly after colour balancing of the same shown in Photo 1-11. The middle two pieces in Photo 1-12, vertically dissected apical parts of the same shoot, are two parts of a pouch showing maximum effect of infection, while the other two appear to be partly affected. An additional point observed by the author was atypical increase in hardness of the apparently non-infected parts (Photos 1-11 and 12), while moving from the anterior to apical side. These effects, however, are qualitative and may be quantified by using the requisite equipment, but the evidence of infection’s moving forward seems non-refutable.

In case of guava, maroon coloured nematode (Saprophyte) was observed in the pith and shown in Photo 1-13 and reported by Idrees et al. (2006a) as well. It is difficult to comment thereon except that it keeps on growing parallel to branches and seems to be tailoring them out in an atypical way as seen from Photo 1-4. For Amaltas, two root sections, i.e. one each dissected horizontally and vertically (Photo 1-16) show the existence of a brown coloured infection therein. This is followed by a vertical dissection of Amaltas’ roots in Photos 1-17 to 1-20, where the first is closest to the stem and otherwise for the last. The extent of disease seems to be the highest in case of sample at Photo 1-17 and almost negligible in the one at Photo 1-20. It is also noted that sample at Photos 1-17 to 1-19 are zigzag, while that one at the last is almost straight. Thus, it appears that the extent of infection and shape of roots are correlated and disease moves forward.

Projections and bumpy out-growths appear in various cases, i.e. mango, Nim (Azadirachta indica) and Shisham (Photos 2-2 to 2-4). In case of several plants, such as Semal branches become oval (Photo 2-1) in stead of being round ones in normal growth process and also different from buttresses. Bulges appear on branch drop out points (Photo 2-5) in case of Semal. Cankers in case of stem (Eucalyptus Photo 2-6) is already reported [Chaudhry and Ahmad (2004)],
while twisted stem with bulges on it [Jangli Toot (Brossonetia papyrifera) in Photo 2-7] is an exceptional case. Photo 2-8 shows Poplar (Populus tremuloides) having two main branches, of which, one is dried and compressed inside, while the other one is green. One half and complete drying of Shisham is seen from Photos 2-9 and 2-10, respectively. A leaning stem of Shisham having a main branch at the right angle is seen from Photo 2-11. The bark, in Arjun (Terminalia arjuna), shows a variety of erratic and bumpy out-growths (Photos 2-12 and 2-13), while hollow stems for Jamun and Eucalyptus are seen from Photos 2-14 to 2-16.

**General features of the main issue**

Khan’s (2004) thesis of treating die-back as a syndrome is a starting point for this study. Further, Gill’s (2004) observation of disease moving upward (or more precisely forward) suggests that die-back is not true. This study recognizes the main issue as an outcome of the GRENE Syndrome, which came to surface with a large scale appearance of partial or complete drying of Shisham in late 1990s in the sub-continent (Pakistan, India, Bangladesh, Bhutan and Nepal) and same issue noted for Eucalyptus in Australia during 1970s.

The pathogens (ENOs) get into xylem tissues as a result of horizontal or vertical injuries to plants, such as pruning, loping, scratching, budding, grafting, etc. [Idrees et al (2006a) and (2006b) and Ellahi (2006)]. It may be noted that Keerio (2006) reported that, in Sindh, damage to grafted mango trees was more than that to the traditional mango variety. Photos 3-1, 3-2 and 3-3 exhibit horizontal injuries to Shisham, Bair (Ziziphus jajuba) and Sarv, respectively, while vertical scratching to Shisham, Eucalyptus and Bakain (Melia azedarach) may be seen in Photos 3-4 to 3-6. The ENOs act both above and below the ground, take a part of raw food and oxygen, release carbon dioxide and other harmful excretions within body of the plant and disturb quality and quantum of food delivered to leaves. All these abnormal activities lead to hollow stem, morphological changes, imbalanced food composition and adverse effects on plant health and its physiological functions.

The ENOs move back to reach main stem and then move forward both above and below the ground to reach apex of roots and branches. They cause death to xylem tissues at a branch-out point, stop the supply of raw food and keep on moving up to the last xylem cell, which leads to dieing, decaying and ENOs spreading out through branches and other parts of the plant [Bakain in Photos 3-8 and 3-9 and mulberry (Morus alba) on right side of Photo 3-9]. Further, movement of infection may be restricted to a specific part or may travel through the infected plant. It is, however, established that infection moves forward either up or down.
Other features of the GRENE syndrome

The ENOs start taxing plant’s raw food and overall requirement for organic matter and its oxygenation is increased, which is scientific and accords with recommendations of the task force headed by Mr. M. Shafi Niaz and that of the Australian biologists. However, it is a temporary measure and not sustainable.

The ENOs may create a variety of bio-chemical and biological exudates in liquid and vapourized forms. Inside body of the plant, exudates have temperature and pressure which is more than that of the outside environment. This causes stem’s bulging out and canker formation, on southern aspect of the tree [Chaudhry and Ahmad (2004)]. After lopping, bulging in of the stem and branches is also noted (Jamun and Shisham Photos 3-10 and 3-11). To explore further, a bulge noted in Arjun in Photo 3-12 was scratched, which showed a very hard canker underneath (Photo 3-12).

Some observations were made about the ENOs’ actions in root system of Mulberry, Amaltas and Jangli Toot. In case of Mulberry, ENOs get developed into hard and aero-shaped structures either themselves or using plant tissues, which keep on penetrating through roots, leaving behind a tunnel, which gets filled up with soil and inner side of the root adjoining soil turns black, but root remains alive and functional because four essential parts shown in Figure 2 remain alive. Scattered scratches outside these roots exhibit termite action, but none were found in a passage of about one foot width covering the roots. In case of Amaltas, roots were in an irregular zigzag shape (Photos 1-14 to 1-19) with hard structure moving ahead and leaving behind a dark brown matter in the pith, which appeared entirely burnt and black near the stem. Contrary to Mulberry’s case, neither scratches were found on roots nor any living or dead insects were seen at all in a radius of about three feet around the stem. Finally, the fruit, containing seeds, is the most refined output of the plant and receives only the manufactured food via phloem and ENOs have no way to enter the fruit and seed remains free of them.

Culture and identification of ENOS

The existence of Ganoderma lucidum by PFRI and Phytophthora cinnamomi by the Australian botanists of the Melbourne University [Jamil (2004)], Fusarium (Bajwa and Mukhtar 2006) and Botryodiplodia species by Idrees et al. (2006a) in affected parts of plants may not be denied. However, to be a causal organism for the instant issue requires that the identified pathogens, after inoculation into healthy plants, must create the effect being researched into. A test of this crucial issue and its outcome are only reported in Idrees et al. (2006a), but that is too restrictive and can not be generalized. Thus, the identification process is dubious and the treatment recommended as a result of this investigation may not be effective. It may safely be inferred that fungi identified so far is a secondary but not a primary cause of the subject matter being dealt with. Hence, it appears that isolated material either does not contain ENOs or environment of the culture process is incompatible with that required by them.

To sum up for policy recommendations

It transpires that main cause of the GRENE Syndrome are ENOs, which are a non-traditional form of infection affecting exposed xylem tissues. The development of curative measures may take a long time, but in the meantime, following short-term protective measure may be adopted:

To avoid damages, slicing, slashing, pruning, lopping, etc. have to be stopped and areas meant for livestock grazing need to be earmarked and the rest has to be declared as protected plantations.

In the case for grafted and budded plants, the sliced branch of the stock plant may be provided wax coating to avoid ENOs’ entry.

In case of Shisham, nursery may be raised by growing seed in small polythene bags, which may be transplanted without stumping.

The process of ENOs’ identification and evolving remedial measures essentially requires growing them in a culture medium/ environment, which is compatible with their natural requirements.

The GRENE Syndrome requires a multi-disciplinary approach for its analysis, diagnosis and to discover the remedial measures.
The said preventive measure may be applied to a very limited extent for the plants grown from seed, but of a little use for a variety of fruit, floral and other plants obtained through grafting and vegetative methods. Hence, recommendation merits top priority.

REFERENCES


Keerio GR (2004). Highlights/Progress of Work Carried out on Dieing/Drying of Shisham and Other Trees In Sindh’, A Paper Presented to the Second National Seminar on Shisham Dieback held on June 29, 2004 at the Punjab Forestry Research Institute, Faisalabad.


Photo 1: Atypical Stems, Branches, Roots and Selected ENOs
Photo 2: GRENE Syndrome Effects on Stem and Branches
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**Photo 3:** Modes of ENOs Entry into the Plant