Review Article
Advancements in Life Sciences – International Quarterly Journal of Biological Sciences

ARTICLE INFO

Date Received: 01/02/2021;
Date Revised: 06/06/2024;
Available Online: 10/07/2024;

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How to Cite:
Aziz I, Ali S, Rehman Z, Aman S, Shahid AA (2024). Microbial forensics: are we going on right path for management of crops biosecurity in Pakistan? Adv. Life Sci. 11(3): 544-548.

Keywords:
Biological terrorism; Microbial forensics; Pathogen; Plants; Crops; Biosecurity

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Microbial forensics: are we going on right path for management of crops biosecurity in Pakistan?

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Abstract

In any country biological terrorism outbreaks on crops could reduce the quality and quantity of agricultural products, which in turn decreases the consumer's confidence, nutrition loss, resulting in worse impacts on the nation's economy, international souks and is subsequently harmful for the environment. Awareness for a crop bioterror incident begins with the start of the threat symptoms, which must lead to the progress of a plan to thwart. However, most of Pakistani scientists and crop producers have not focused on the option of deliberative plant pathogen introduction but instead they use their expertise for the prevention of natural introductions and the improvement of cost-effective strategies for disease management. It is also an interesting fact that under the mask of genetically modified feeds, high yield varieties and hybrids; we are also importing bio terrorism. Are we going on the right path? So, it is time that we should review the global microbial knowledge and should apply it according to our needs. In ongoing topics of present article, we will review the microbial forensics status globally in comparison with Pakistan. We will also suggest how to fill these gaps.

Introduction

Microbial forensics is the discipline which is applying the scientific methods for analysis of bioterrorism and/or accidental release of a biological agent and toxins. In present review the microbial forensics under the headings; Vulnerability of Pakistani crops and status of non-indigenous and exotic pathogens, Global history of exotic plant pathogens as bioweapons, Role of microbial forensics in crop biosecurity, Special measures for microbial forensics etc. will be studied.

Methods

Literature search strategy and selection criteria

Global work with the keywords" Microbial forensics, plant pathogens, bioterrorism, management" was searched. Inclusion criteria is "plant pathogens" only, all other pathogens were excluded. Plant Pest Forecasting System given on the web add, (http://www.danforthcenter.org/iltab/Geminiviridae/ and http://gemini.biosci.arizona.edu/), National Center for Biotechnology Information (NCBI) (http://www.ncbi.nlm.nih.gov/entrez/) and the PathPort/ToolBus framework were searched.

Discussion

Vulnerability of Pakistani crops and status of non-indigenous and exotic pathogens

Agriculture is an indispensable component of Pakistan's economy. It currently contributes about 18.9 percent to GDP while absorbs 42.3 % of labor force [1,2]. Microbial forensics is still new to Pakistani science [3]. Regarding vulnerability of crops, usually the phrase "unfavorable weather conditions" is used in all related documents. The performance of the agriculture sector remained connected to said unfavorable weather conditions, which resulted in lower production of crops. Neither any type of microbial attack nor other pathogenic condition is mentioned usually. While at global level, the reasons of losses are searched scientifically [4]. For example, if in the United States alone, plants are subject to attack by different non-indigenous pathogens, it has been reported that the exotic factors were primarily fungi, viruses, bacteria or nematodes [5]. If we also ponder the problem and put record of any microbial eruption, then it could be decided that either it is caused by the planned release, natural or engineered biological agent. So, it is important to know that in agricultural countries like Pakistan, a deliberate exotic pathogen introduction could results in:

(i) Low crop yield and quality
(ii) Hunger
(iii) Significant public shock and panic due to loss of confidence in government
(iv) Political disruption
(v) Negative impact on country's economy and international market

Global history of exotic plant pathogens as bioweapons

The use of exotic plant pathogens as bioweapons is not a new idea, following are the examples of the biological warfare use [6, 7, 8, 9, 10];

1. During first and second world war Germany programmed and researched it
2. The United States conducted research involving a number of pathogenic microbes, including the biological agents causing anthrax, foot and mouth disease, and rice blast
3. Former Soviet Union conducted programs from the Second World War through the Cold War
4. Beginning problem of the Iran-Iraq War.
5. Syria gas victims, 2013
6. Countries are thought to have biological weapon programs are Canada, France, Japan, and the United Kingdom.

Although there has been no documented case of the deliberate usage of microbes to attack crops. However, a posture of vigilance dictates that reasonable steps should be taken to ensure that crop biosecurity abilities "before" an upsetting event occurs not "afterwards".

Role of microbial forensics in crop biosecurity

Now, if the case is deliberate introduction of microbes as bioweapons or accident arrival in a country, importance should be given to the source. The method, time of microbe's introduction and the transducing agents which are responsible for microbe's arrival should be analyzed. This study falls in forensics as well as epidemiology [11-18]. Forensic science deals with scientific methods for searching law violations in both civil and criminal matters [19-25]. For preservation of public safety and confidence, forensics also investigates each segment of microbial discrimination like from sampling to shipping.

For making decision that whether a certain case is related to endemic intrusion of pathogen, or it is bioterrorism, the responsibility of microbial forensic scientist get enhances as compared to normal situation. A microbial forensic scientist should be quick in answering the following questions;

1. The first question is related to the time between the intrusion of a pathogen and its recognition.

2. The second question is related to establish a proper protocol with specific time limit to reduce the interval between sample collection and its arrival to the laboratory.
3. The third question is related to the time required by forensic scientists for laboratory assessments.

In the present article, we propose the integration of the traditional knowledge of plant pathology and the advanced field of forensic science, envisioning the birth of the new field; microbial forensics. We designate the possible threats to plant resources and development of national security plan for microbial forensics that addresses crop-microbial bioterrorism. For achieving this goal, we review currently existing information and technologies. We also give recommendations for serious research, advancement of new technologies, substructure, and distribution of human and financial resources to ensure a strong competence in microbial forensics.

Special measures for microbial forensics

1. Case history

Making a case history for start of work in case of microbial forensics is same as the casual forensics scientists are working. But some specific steps are required to add. For example, the knowledge of exotic disease, its host's information, suspected pathogen and microbial profiling. Along with determination of all the regular steps of forensics profiling e.g. determining the forensics challenge, trace evidence, geographic profiling, criminal profiling, DNA and computer profiling are included but with some specific changes. Mistakes made during the first step of this process, e.g. sampling or disease identification, cannot be justified at the later stage of the investigation. Application of forensic methods to plant microbial terrorism requires specific steps in making a full-fledge strategy.

2. Geographic profiling

Careful documentation of disease characteristics and sampling the specific crime scene gives a clear picture for geographic profiling. For achieving the exact goal, on site disease assessment in the field should be done before any disturbance of the site. For preserving the pattern of disease or any other unusual characteristics, satellite image should be taken. Global positioning system (GPS) technology could also be utilized for demarcating the symptomatic plants.

For the purpose of microbial forensic collection following instructions [19, 20, 21, 22, 23, 24, 25] could help definitely in obtaining the correct results:

- Standard Operating Procedures (SOPs) should be varied according to the collection style, method, crop species, microbe specie.
- Containers for collecting the samples should be clean and new.
- The forensic samples should be collected directly into the container.
- Documentation of administrative log should be done.
- Detailed information about the place, chain of custody, a collection site map (could be helpful in repeating the sampling from the same location).
- Transferal documents for laboratory, details of crop, field history, photographs of crop and field layout should be well documented.
- Depending upon the phytopathology *e.g.* narcotic lesions, affected area of plant, biochemical changes in different parts of a plant, following are the specimens of different forensic field sample types:

  a. Whole plants
  b. Selected plant parts
  c. Plant surface swabs and exudates
  d. Soil
  e. Root tissue
  f. Suspected insect vectors
  g. Natural water near to field
  h. Irrigation water in or near the fields
  i. Air samples
  j. Biological samples (weed hosts and soil/ aquatic organisms log, the complete chain of custody, a collection site map(s)

Sample collection pattern

For detailed mapping about microbial disease incidence, it is important to have a clear view on severity of disease. For achieving this objective, concentration should be given to preparation of clear pattern of sample collection. Although W and X-shaped patterns are in use for sampling but Delp *et al.* (47) advocated the usage of a stratified random sampling pattern. In which following logical steps will be observed:

1. The field should be first divided into several strata (*e.g.*, regions of higher or lower disease risk)
2. For reduction of personal error (as observed in W and X-shaped), the random collection of samples within each stratum should be done.
3. The sampler must be alert of the fact that there should not be a single point of inoculation so that deliberate release of

microbes by aero plane may result in line/area sources of inoculum.

Logistics

Transportation or logistics is another important step in microbial forensics. Following steps are recommended [26]:

1. A two-person team is enough to work 6-8 hours (including travel) in the field for collection and documentation of 30- 40 samples.
2. Sample numbers should be found in the framework of the analytic and volume facilities of the forensics laboratory.
3. Any tampering (related to the time lapse between collection and receipt of the samples) could be avoided by the use of custody seals which will alert the recipient.
4. All steps/ information must be documented, exclusively when samples are obtained from different fields or locations within a field.
5. Decontamination of workers and apparatus is important to avert the investigating person from becoming a vector, spreading the microbes and causing false positive results by contaminating the microbe-free samples.

Sample storage

Before analysis, long term storage of microbial samples is an important step in forensics [26]. Great care is recommended. Depending upon the viability of plant pathogen (because some microbes are more viable than others), the storage of leaves, seeds or fruit should be done carefully. Following measures are recommended in this respect:

1. Environmental conditions should be documented during storage
2. The record of chain-of-custody should be well maintained, especially the times visited by the persons who have direct access to the samples.

First finders and first reaction

Crime scenes in human forensics is visited by "First finder" the person who found the crime first and the "first reaction" is normally from the police. But in case of microbial forensics, first finder is the plant grower or crop consultant, and the first responder is generally the government official or the researcher of microbial forensics lab. Instant and timely management of crime scene is possible only if both persons should have prior knowledge and skill to understand the mode of crime (26). Networks are available in western countries like National Plant Diagnostic Network (NPDN) (http://www.npdn.org/) which provides trainings but in case of countries like Pakistan no measures are taken still, probably due to lack of funds.

Now to onward we will discuss some advanced approaches which we should adopt in countries like Pakistan if we want to combat the bioterrorism.

Use of epidemiological models for forensic analysis

According to American researchers [26] forensic detection from plant microbes to their sources needs research from plant to the landscape. For understanding this philosophy different epidemiological models are adopted [26]. These models help in quantification of the microbe within the plant/ field or landscape and estimation of initial infection time depending upon the environmental conditions and growth rate/stage of the host.

These epidemiological models also help in understanding the spread of disease to neighboring crops, based on environmental situation and vector activity. So basically, there are two epidemiological tactics supported by the said epidemiological tools:

i. Proactive approaches: help researchers to understand geographical variations and its impact on disease

ii. Reconstructive approaches: enable understanding the microbial incidents and their aftereffects

Following are the epidemiological tools suggested by different researchers [26] for achieving a clear view of proactive and reconstructive approach:

(i) **Climate matching**

Climate matching is a proactive epidemiological tool which helps identifying the areas where unusual disease events potentially occur and its probability for establishment in the specific area and rapidity of spread [27].

(ii) **Bioclimate envelope**

A pragmatic "bioclimate envelope" of microbial environmental need could be observed based on its short- and long-term distribution and this information is used for identification of geographical area which match the specific microbe's requirement [28].

(iii) **Software tools**

Multiple software are used as understanding the presences and patterns of crops in the world like:

- Climex [29, 30, 31]
- FloraMap [32]

(iv) **Web-based modeling systems**

Use of web-based modeling systems is a retrospective approach. When the knowledge of environmental requirements of microbes has been approached experimentally, web and weather-based disease models can help to define its probable locations of establishment [33, 34]. North Carolina State University used a web-based method, APHIS Plant Pest Forecasting System given on the web address, http://www.nappfast.org/index.htm, that links meteorological plus crop distribution databases with geographical information system (GIS). The system gives information about modules for many pests, modifications of pests and exotic plant microbes [35]. DYMEX is another example. This simulator is developed in Australia [36]. This retrospective approach helps in determining the relation between whether conditions favorability at a suspected release site with a given time of infection and development of disease [37].

(v) **Trajectory analysis**

This approach could be used in both proactive and reconstructive ways. Trajectory analysis tracks airborne microbes in actual time. Air parcels pick up spores from source areas and transfer them to upper-air levels from which the information about their distribution at distant locations could be suggested. So the models of atmospheric dispersion e.g. HYSPLIT4 (Hybrid Single-Particle Lagrangian Integrated Trajectory) [38] could be used with proactive approach to predict the seasonal movement of air born microbes and with retrospective approach to identify the outbreak source. GIS, GPS and other remote-sensing platforms have been applied in plant forensics [39, 40, 41].

So, on the basis of given knowledge, a three-tiered approach could be summarized consists of:

- Comparison of aerial and satellite images before and after microbial attack for disease assessment
- The field-based assessment of disease severity
- Integration and mapping the remotely sensed and field-based data

(vi) **Crop phenology**

Data derived from ground surveys, remote sensing and crop models, provide critical forensic evidence *e.g.* time of infection while crop phenology gives information for stage of infection [42]. It gives information for leaf age, position and lesion development [43].

Microbial Forensic: past and preset

Under this heading a complete scenario of microbial forensics global status is given for clearing our pathway towards advancement in this field. For this we reviewed microbial forensics form different angles, as given under:

Identification methods

From a forensics point of view, the first question after outbreak of a disease is about the natural or intestinal occurrence. The exact answer depends upon the information about the presence or absence of microbes before the occurrence of disease. Till now the evolution of different plant pathological techniques reaches molecular typing but it helps in quantifying the disease effectively only after occurrence of disease [26].

Comparative interpretation

Three tire categories have been used since past to present in microbial forensics, as given under:

- Inclusion criteria (originating from the same source)
- Exclusion (not originated from same source)
- Inconclusive originating (having variations)

Evolution in forensics typing methods

Multiple methods of Microbial forensics have been used by plant pathologists in the past. However, in the present time both nucleic acid-based methods and non- nucleic acid-based methods are in use. The selection of microbial typing method for forensic analysis is normally based on following characteristics [26]:

(i) Universality *i.e.* one method could be used for typing all organisms of a taxon
(ii) Sensitivity *i.e.* should have high percentage of actual positive results as compared to false negative results
(iii) Specificity *i.e.* should have correct identification of difference between actual positive and false negative results
(iv) Efficiency *i.e.* high percentage of correct test results
(v) Reproducibility *i.e.* consistency in results
(vi) Resolution *i.e.* the degree of resolving power of results should be high

Non-nucleic acid-based methods

There are traditional forensics assays in which molecular biology has no direct relation, but they are still useful. Different assays have been in use since long ago for identifying a microbe by mode of action, symptoms, hypersensitivity, colonial morphology and host range [44, 45]. BIOLOG is a modified assay which gives information about metabolic profiles while

microbe's fatty acid profile could be understand by fatty acid methyl ester (FAME) assay. In both assays profiles of the test strain are compared for the close match with that of given in different databases. Not only bacterial profile but fungi having complex life cycle, could be identified by different assays *e.g.* mating assay [46, 47]. Serological techniques like ELISA and antibody florescence test [48, 49, 50] are also in use.

Nucleic acid-based methods

Identification by nucleic acid- based method is basically dependent on "molecular signature" of microbes. Molecular signature of each microbial species is the unique character of its core genome and consists of basic genes which are important for survival and specific to every species *e.g.* housekeeping gene. As core genome is not affected by environment, neighbor-niche species and host variability as compared to flexible part of genome (antibiotic resistant gene harboring area) so different molecular techniques are designed to inspect it. Restriction fragment length polymorphism (RFLP), phage typing, DNA fingerprinting, DNA probe analysis, genetic marker analysis, sequencing of specific genome region such as 16SrRNA, Polymerase Chain Reaction and complete genome sequencing are useful molecular biological methods used equally for bacteria, virus and fungi [51, 52]. Adaptations in these methods are quite enough to check the mutations, multiple strain/species infection, changes in virulence due to difference in natural and artificial lab media. Genome dynamics and phylogenetic studies have also importance when there is matter of extra-chromosomal elements, mitochondrial DNA, human modifications, plasmids and relation to pathogenicity [53].

For answering the question that how molecular markers are selected for microbe discrimination and forensics, following are the ways which give us clues:

i. The selected molecular marker should be inherent which are unlikely to change over time so the suspect and crime scene microbe could be separated.
ii. In case of human, DNA typing of the selected molecular marker is based on the knowledge of allele frequency, the number of loci and inheritance mode. So, the autosomal markers are preferred due to genes presence on different chromosomes and meiotic homologous recombination among markers at distance on the same chromosome which resulted in accuracy in frequencies of the markers in the population. Same approach is used with bacteria, viruses and fungi, on same piece of DNA or RNA for avoiding the frequent recombination [54].
iii. Keeping in mind that microbes evolve much faster than humans so the degree of recombination which varies among species is also important for selection of molecular markers [55].
iv. Comparison of multiple sets of isolate pairs whose neutral mutation rate and frequency of difference at neutral sites is known from the world record, could be used as molecular marker for viewing the difference at neutral sites between suspect microbe and a crime scene microbe. Which allows the calculation of the time of divergence between two types of microbes, its inclusion in case if time scale of divergence is in window and its exclusion if time scale of divergence is beyond the window [56].
v. Different databases are present which help in preparing the peer review note on the background occurrence of microbial attack and characterization of molecular marker. Best example is of an extensive work with reference to *Geminiviridae* (http://www.danforthcenter.org/iltab/Geminiviridae/ and http://gemini.biosci.arizona.edu/). National Center for Biotechnology Information (NCBI) (http://www.ncbi.nlm.nih.gov/entrez/) also helps in searching the relation between new sequence entries, host, microbe, its geographical location and selection of molecular markers
vi. Genome microarrays and subtractive hybridization are also good approaches for marking in forensics [57].
vii. Not only selection of molecular marker but also way of action and knowledge of investigator also matters for forensics investigation. As mutation per cell division also varies per genome. So investigators should not relay on characterization of cloned pair of microbe (suspected and crime scene) only but also have full awareness on microbial behavior. For example, viruses have different evolutionary patterns [58], some RNA viruses e.g. influenza virus evolve rapidly, the evolution rate of *Tobacco mosaic virus* is slow [59] while DNA viruses with large genomes evolve more slowly [60].
viii. In case of bacteria, the common molecular markers are 16S rRNA, spacer in 16 and 23S rRNAs, *groE* and *recA* genes [61].
ix. Comparison of the sequences of multiple genes, whole genome and amplification of

DNA fragments in microspheres followed by pyrosequencing [62] are proving useful molecular markers.

x. Fungal retro-transposons prove a potential set of gene enough for discriminative analysis [61].

xi. Hot spots in a gene are points where frequency of mutation is comparable with that of reference and prove a good marker for genetic analysis [63].

xii. Molecular clock for the rates of deletions, translocations and inversions of repeated sequences is also proved effective in forensics analysis [26].

xiii. Along with the repeated sequence analysis some other markers like the movement of mobile, prophage invasion, the acquisition and/or loss of plasmids proved useful in forensics [64].

xiv. Preparation of phylogenetic trees on the basis of information taken from any molecular technique like PCR solves the problem of identification of very close relation between crime scene microbes [65].

xv. DNA repair system differs in organisms. So, the calculation of time of nucleotide sequence change gives useful clues [64].

xvi. Microbial gene expression and post translational changes can provide a fingerprint of the microbe. For example the complement of proteins resulted from the transcription and translation of a microbe's genes, the phosphorylation of some protein kinases, specific cleavage of a large poly-protein into individual proteins, transcription of coat protein from unique mRNA (in some viruses), glycosylation of viral proteins before virion formation via Golgi membrane systems and mode of action of microbe in modifying the host driven process are good fingerprint informers in forensics [66, 67, 68, 69].

xvii. In host-pathogen interactions, proteins are secreted exclusively in bacteria and is informative about process of pathogenicity [70]. Profiles of these secreted proteins are different between virulent and non-virulent strains and are important in forensics [71].

xviii. Integration of molecular markers-based data into bioinformatics software is also in vogue for forensics [72].

xix. Beyond the molecular approach there are thousands of phenotypic traits e.g. structural changes in sulfur, phosphorus, nitrogen and carbon etc. which give clues for forensics [72].

xx. Different biosensors like plasmon resonance, acoustic and calorimetric also gives forensics knowledge beyond the molecular markers [72].

What to do next: Strategy for Microbial Forensics

Microbial forensics is the field of integration and utilization of diverse types of data. Like fast evolutionary process in microbes, technologies are also evolving rapidly. So, scientists could compete the bioterrorism only by refining the strategies and designing the integrated network of technologies. Following are some points for designing the strategy for combating the bioterrorism by utilizing the microbial forensics:

Curation of molecular biological data via bioinformatics tools

The instrumentation and tools that produce high steadfastness of data about the molecular parts of living cells (DNA/ mRNA/proteins/ metabolites) could be used as molecular signatures or fingerprints. Bioinformatics is the new emerging field and is proving helpful in resolving the gaps present in biological data. GenBank is a good example of that support. But not "all the data" which has importance in microbial forensics, could be still provided by any accessible database. For filling these gaps with reliable data on these databases requires curation. The architecture of these databases could be modified exclusively keeping in mind the needs of microbial forensics. A web of knowledge could be made with the help of reliable organizations distributed in whole world. So, responsibility for such curation could be distributed among specialists of these organizations. Pathogen Portal (PathPort) project is a model project for such effort [73]. It contains the microbes' information and consists of advanced curated data sets, referenced from 50 top graded pathosystems and scientific literature [74].

Maintenance of appropriate security for the information

For answering the question that how the appropriate security for the information presents on databases could be maintained, national biosecurity measures should be well defined. Web services could provide this security via models having security codes and individual usage. One example of such security is WS-Security/ OASIS WS Security TC standards for America. There should be competing databases which not only prove useful for increasing the reliability of data but also provides a competitive environment for maintaining the security.

Building long term strategies

First step in building long term strategies is participation of all stakeholders of biosafety i.e. microbiologists, pathologists, forensics experts, academician, molecular biologists, forensic scientist, law enforcement officers and intelligence officials for fulfilling the information gapes. The next step should be maintenance of this framework of partnership. So, all together; the forensics databases, molecular tools, microbial culture specialists and state's shareholders could set the disciplinary boundaries of microbial forensics.

Combating financial hurdles

Although it is easy to start the energetic program of microbial forensics with all the basic necessities mentioned above, prolong financial limitations over a period of years could lead to momentous understaffing in extension. Cutbacks in academic programs for other finance demanding programs of state and an increasing emphasis on fundamental rather than field-oriented research is producing a serious decline in the number of graduate students who are untrained for field applications. So funds should be released by the state especially for plant microbial-forensics graduate programs. Scholarships for Ph.D. program could be offered in the field of microbial forensics to those students having background of this field with long term strategy. These scholarships could be offered in the form of advancement of different steps and branches of microbial forensics; from basics to advanced steps.

Our master growers are the best source of training. Their expertise could be assigned in state training programs. Eco-feminism may also provide help, because in our fields of cash crops e.g. cotton, wheat and rice, female are the only workers who are nearer to the land and crops from bowing to the cutting. So, viewing the changes in the fields and crops with these female eyes give definitely a big picture. The knowledge of these females may provide a basic source for understanding the geographical-microbial forensics for avoiding the bioterrorism.

Not only for the reason that exotic microbes should be handled and preserved carefully but also for combating the shortage of resources the microbial forensics laboratories could be built in the areas where no agriculture and host plantation is favorable, and land is vacant.

Our crops, rangelands and forests are the target of terrorism. Bioterrorism should be taken as a serious issue by state and model programs like the establishment of a single federal coordinating body, as of United States of America has e.g. a National Center for Plant Biosecurity (NCPB), which is responsible for crop health and is linked to American Phytopathological Society and a number of other scientific societies (http://www.apsnet.org /members/ppb/PDFs/CenterProposal_Final.pdf) could provide leadership and coordination by documenting, monitoring, and protecting our crops/ forests and rangelands against new/ emerging plant microbial diseases. International forums could be arranged for sharing expertise and ideas. Regular forums of such type could help us to get more knowledge in this competitive field.

Suggestive points

The event of Bioterrorism might not be recognized as such. The background information of the affected area is important for deciding that this particular case is either of terrorism or any endemic pathogen is involved. So following hierarchy of information should be strong;

a. Awareness of crop producers about its area's endemic pathogens.
b. Awareness among crop producers about the microbial forensics' challenges by using various media supports *e.g.* television, meetings, books, brochures, newspapers *etc.*
c. Streamlining the emergency information system between effected area, the crop producers, governing bodies and laboratory.
d. Microbial forensics laboratory should be a compulsory unit of forensics laboratories of a country with proper equipment and trained staff.
e. Fair and swift announcement of conclusive results should be commended. So that plant producers, governing bodies and related persons could take microbial forensics as a serious issue and liable to answer the query that whether the criminal activity has occurred or not.
f. Establishment of new tools specific for microbial forensics.
g. Filling remaining gaps and the required capabilities to plug them, which will serve as the basis of the development of forward strategy.
h. Critically assessment, selection and sharpening of existing methods, standard operating procedures (SOPs) and protocols should be surfaced so that a reliable bridge of planning and work be formed between plant producers, pathologists, microbial and molecular forensic scientists.

Conclusions

Our crops, rangelands and forests are the target of terrorism. Bioterrorism should be taken as a serious issue by state and model programs like one example we have given in this review could address both prevention and alertness. Only practical approach of microbial forensics could help us to determine the source of terrorism. So, our strategies should be (i) stringent, (ii) able to point to the source, (iii) sufficient to reach the source and (iv) evident for applying laws of state. Investment in the new field of microbial forensics *i.e.,* research, structure, training and planning is the need of time to secure a frontline nation.

Author Contributions

Worked and written by Dr. Ishrat Aziz.
The remaining authors contributed to revising the manuscript.

Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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