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Assessment of *Azadirachta indica* Die-back disease recovery and damage from Narayanapet District, Telangana State

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The current report reveals the assessment results of *Azadirachta indica* Dieback disease in the Narayanpet District of 12 locations as mandals, Telangana State, India. The die back disease has damaged hugely in Telangana State. In the present documentation Narayanpet Dist. was selected as study area. A total of 149 sample trees were selected and assessed regularly for one year. On the basis of assessment of results among 149 trees large trees were 59, medium trees were 46 and small trees 37. The recovery rate was also determined with frequent visits which revealed that the recovery at branches was 54, from side branches 72 and terminal buds are less with 17 in number. The terminal buds damaged are the initial sign of the disease as results with 103 trees recorded and the results of the parts the disease symptoms are recorded 39. The fungal infection of tree trunks were also assessed. From the diseased trees the pathogen was isolated and described. The current report will be very useful to the upcoming researchers.

Key words: *Azadirachta indica,* die-back disease, recovery and damage, Narayanapet District,

INTRODUCTION

The Neem tree and Indian culture have a close relationship that is unbreakable. Commonly referred to as “Indian lilac” or “Margosa,” it is a member of the Mahogany family and is widely spread throughout India. Given that it is sacred in Indian culture, it is regarded as significant. In India, there are over 20 million trees, of which 5.5% trees are in Karnataka, which ranks third (Venugopalan and Visweswaran, 2013). A neem tree shows a wide climatic range, topographic adaption, and superior stress tolerance as compared to other factors and other species (Ghasolia and Shivpuri, 2004).

The tree is shown as a cure-all. In India, the various neem tree parts are used for adaptable medications. It is widely used in Ayurvedic medicine. Non-wood items like flowers, fruits, nuts, oil cakes, leaves, bark, and gum are valuable in addition to strong wood (Sateesh, 2004). 40% of the seeds’ deep yellow fatty oil , the typical “Margosa Oil,” is produced, which has therapeutic effects, and is used to treat sprains, ulcers, leprosy, rheumatism, and chronic skin conditions (Ghasolia and Shivpuri, 2004).

The tree’s seed cake is readily available and effective as fertiliser. Neem species have been found to have insecticide and pesticide (195 species) characteristics. The tree is thus known as “Village Pharmacy,” “Doctor Tree,” “Wonder Tree of India,” or “The Bitter Gem” in the area. Despite being so well known for its antibacterial and other biological versatility, fungus diseases attack Neem trees. (Zeenat ,2018). *Phomopsis azadirachtae* is the culprit behind neem’s die-back. Irrespective of the age, size, or height of the tree, the fungus distresses the leaves, twigs, and inflorescence. It has been observed that in persistently infected trees, it has nearly invariably led to a 100% loss of fruit production, which has

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impacted the availability of a highly valuable source of botanical pesticide, the seed. The disease needs to be rapidly managed as it is spreading at an alarming rate. There have been fungal reports from the Mysore area in the past (Mahesh *et al*. 2005). In Telangana state, the disease symptoms were only recently reported.

The population of neem trees has been harmed in recent years by fungal infections that are affecting the tree and spreading from district to district. The current work has successfully provided the assessment information of *Azadirachta indica* Dieback disease damage and recovery from the Narayanpet District of Dhanwada, Kosgi, Krishna, Maddur, Maganoor, Makthal, Marikal, Narayanpet, Narwa, and Utkoor.

MATERIALS AND METHODS

Disease survey

A disease survey of the neem Die-back illness was conducted in the Narayanpet District of Dhanwada, Kosgi, Krishna, Maddur, Maganoor, Makthal, Marikal, Narayanpet, Narwa, and Utkoor (Fig.1). Each sampling area had an average of 15 neem trees, and the locations of both damaged and healthy neem trees were mapped using the Global Positioning System (GARMIN GPS12). The height, GBH, age, latitude, longitude, altitude, and degree of disease of each individual tree were recorded (Sateesh *et al*. 2004).

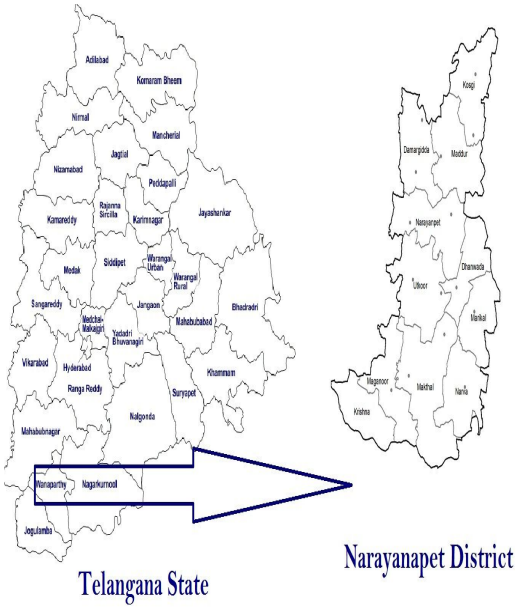

Fig. 1: Study area, Narayanapet district, Telangana state, India.

Isolation of pathogen from twigs

The die back affected neem terminal buds were bought to the laboratory, each twig had a middle transition zone and was trimmed to a length of 2-3 cm. Twigs with and without illness were washed under flowing water from the faucet for 1 h. They were also cut into short, 1-1.5 cm segments with a transition zone in the middle. Segments underwent a five-minute surface sterilization with 4% sodium hypochlorite and six to eight sterile distilled water rinses. MEA supplemented with 100 ppm chloramphenicol were plated and segments were placed. Plates were incubated for 7-8 days at 25°C with 12 h of darkness and 12 h of light alternated (Prasad *et al.* 2007).

Data analysis: The documented information compiled, with graphical analysis has recorded.

RESULTS AND DISCUSSION

Die-back of Azadirachta caused by *Phomopsis azadirachtae* was first reported from young forests of Dehra Dun, North India (Fathima, 2004) and subsequently by Girish and Bhat (2008). The signs of disease are fruit rot, inflorescence blight and twig blight among others and currently it is the most serious and deadly illness of neem tree regardless of its age, size or height (Zeenat, 2018). Though it can be seen all year round, the condition is more noticeable from August to December. The onset of the rainy season marks the beginning of the symptoms, which develop in severity during the rest of the rainy season and into the first few months of winter. Year after year, the disease causes the tree's mortality to proceed. The main sign is twig blight (Fig. 2). Additionally, the disease causes fruit rot and inflorescence blight, which together reduce 100%.fruit output. The main areas affected are the terminal branches. Conidia, which are carried by insects and raindrops, are main cause for disease to spread by seeds. Although conidia are fertile and easily germinate, germination of conidia has not been observed *in vitro* (Sateesh *et al.* 2004).

P. azadirachtae, a Deuteromycetes fungus, is the cause of Die-back on neem. All of the twig explants produced from sick neem trees were successfully separated from the pathogen using PDA media. Although teliomorph (*Diaporthe*) was described in many *Phomopsis* species*, P. azadirachtae* was not connected with it. Despite cultivating the pathogen

on specified medium and under precise conditions required to produce sexual phase, it was not possible to induce teliomorphic or sexual phase in *P. azadirachtae.* There is no known collateral host. Both Alpha and Beta- conidia spores are generated by the pathogen.

Mycelium submerged, branching, septate, abundant, colourless, subsequently turns pale brown, is how the pathogen is described. Conidiomata pycnidial, solitary or aggregate, half-immersed, pale brown to dark brown or black, ampuliform or subglobose, unilocular, thick-walled, textura annularis, uniform throughout with the endogenous basal swelling cone with lumina of bigger cells, outer layers melanised, 300-500 μm high, up to 900 μm wide in sections, very short basal clypei, ostiole. Conidiogenous cells are phialidic, subulate or filiform, integrated or discrete, channel and collarette minute, hyaline, periclinal thickenings of variable thickness, 5-8 x 1.6-3 μm, producing both alpha-conidia and beta-conidia, conidia acropleurogenous. Conidiophores are simple or branched, short or elongate, septate, filiform, hyaline, and Beta-conidia are hyaline, filiform, hamate, eguttulate, aseptate, 16–25.6 X 1.6–2.0 μm, and have unknown germination. Alpha-conidia are hyaline, fusiform, straight, 2-4 guttulate, smooth, aseptate, and 4.8–11 X 1.6–3.2 μm in size (Fathima *et al.* 2004).

Mycelial development was found to be unaffected by light. Sporulation needs high relative humidity and adequate light, which should last 8 to 12 hours per day. The temperature where vegetative *P. azadirachtae* can grow in a wide temperature range, from 10-35^0 C, and its optimal growth temperature is between 26 and 28 ^{0}C. Ideal pH was determined as 6 and ranging between 4-9. The best carbon sources were discovered to be sucrose and starch out of eleven different sources, including cellulose, fructose, galactose, glucose, maltose, mannitol, lactose, sorbitol, and sucrose.

The pathogen preferred potassium nitrite and ammonium sulphate over other nitrogen sources such urea, asparagine, glycine, potassium nitrate, sodium nitrite, and asparagine. *P. azadirachtae* thrived on media that had been supplemented with thiamine, riboflavin, nicotinic acid, pyridoxine, and other vitamins including pyridoxine, riboflavin, biotin, and inositol. Mycelial mat to fully develop up to 90 mm needs roughly 8–10 days. After 15 days, sporulation begins in a petri dish. The ability of a pathogen to thrive in a variety of physical environments and ingest different chemical elements demonstrates this ability to withstand changing environmental conditions (Sateesh *et al*. 2004). On all of the tested substrates, aerial mycelia persisted for around 18 months at room temperature. Between 21 and 36 months, different media had varying degrees of mycelium viability under refrigerated conditions.

The growth of the pathogen in MEA medium is depicted in Fig. 3.The kind of media and storage conditions also had an impact on the viability and germination of conidia. With special emphasis on Potato Dextrose Agar (PDA) and Malt Extract Agar (MEA), conidia could be kept alive for up to 24 months at ambient temperature and up to 36 months in the refrigerator (Sateesh *et al*. 2004). The ability of the fungus to maintain conidial and mycelial viability for a comparatively long time in various environmental circumstances may be the cause of the disease's recurrence in consecutive monsoon seasons (Fathima *et al*. 2004). Seeds are used to spread *P. azadirachtae*. Thus, it spreads from seed to seedling and may cause a large-scale disease outbreak. Studies on the pathogen's ability to spread through seeds showed that *P. azadirachtae* had a long history. The embryo, cotyledons, and seed coat all contained the infection. Other than this, many other fungi, including *Aspergillus ochraceous* were isolated from seeds such as *A. penicillium*, *A. flavus*, and *Fusarium oxysporum*. Embryos only contained *P. azadirachtae*. According to studies on the transfer of pathogens from seeds to seedlings, the pathogen can cause seed rot, seedling rot, weak seedling growth, as well as seedlings with fibrous root systems and those without root systems (Girish and Bhat, 2008).

We tried conidial inoculation, mycelial inoculation, and toothpick inoculation. The pathogen might be established in the neem plant using the conidial inoculation technique. Twig blight, a Die-back disease sign, emerged as a result of the pathogen's establishment. The twigs of all the neem plants that had been treated with conidia contained the same fungus. The pathogen's constrained host range was revealed to *A. indica,* when it failed to infect *Melia azedarachta*, an associated taxon of the same family (Girish and Bhat, 2008). The results provided insights of an assessment of the recovery and damage of neem (*Azadirachta indica*) tree causing dieback disease

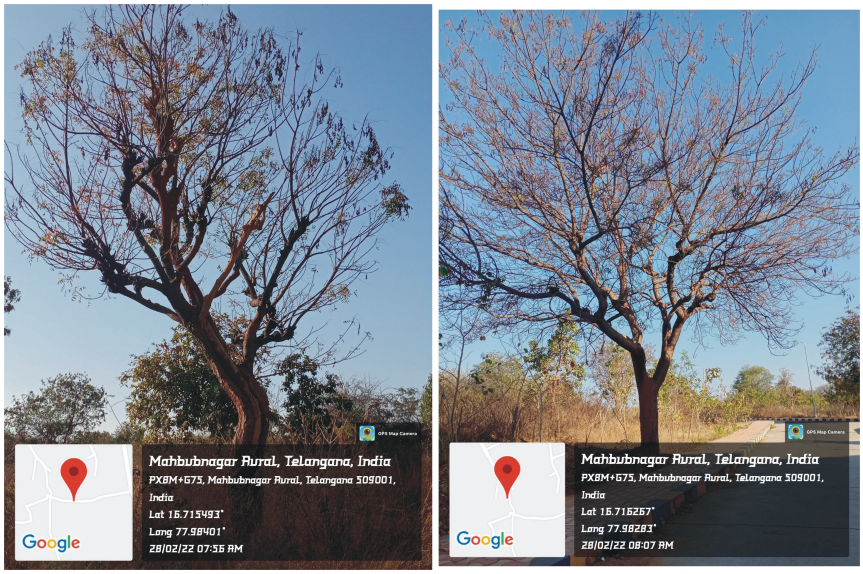

Fig. 2: Die-back disease of neem trees from study area

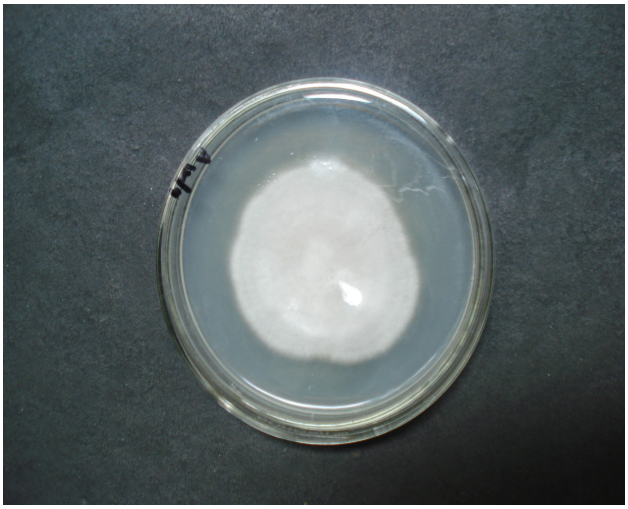

Fig.3: Mycelial growth of dieback pathogen *P. azadirachtae* on MEA medium

by *P. azadirachtae* at the selected study area of Telangana State, India. The Dieback disease of *A. indica* has severely damaged the population at Telangana State. The study area is one of the rural locations of the district Narayanapet (Fig. 1), it covers the all the types of environment conditions. The district was chosen as the emphasis of the current documentation. A minimum of 149 experimental trees were chosen from 12 locations of all mandals of the district, and they underwent a year of routine evaluation. Based on evaluation of the outcomes among 149 trees there were 59 large trees, 46 medium trees, and 37 little trees. Where the healing rate is also discovered by regular visits, it is found that there are only 17 terminal buds, 54 branches, and 72 side branches recovering from

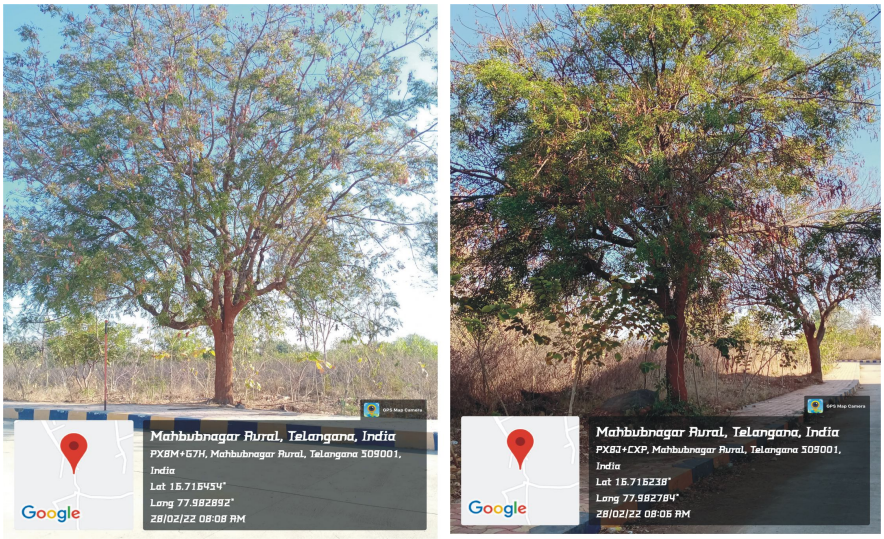

Fig 4. Die-back disease recovered neem trees from study area

damage. The disease's first symptom is the destruction to the terminal buds, according to results from 103 trees recorded and the results of the parts the disease symptoms are recorded 39. The fungi infection with tree trunks also assessed. From the diseased trees the pathogen was isolated and described. Ultimately by the month of June 2022, almost all trees were recovered. Naturally the 98% percentages of trees were recovered naturally in the study area (Fig 4). The natural observation, assessment results can reflects the recovery of forest and biodiversity areas of Telangana State.

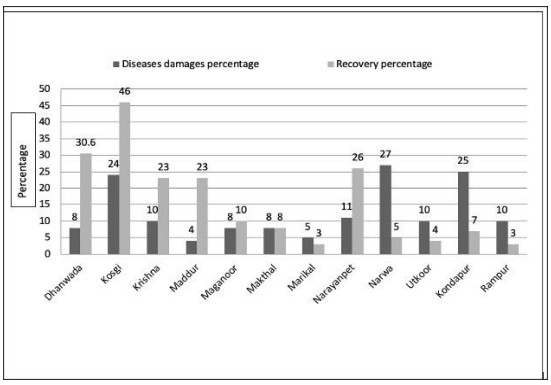

Fig.5: Disease damage and recovery percentages of neem trees in the different loctaions surveyed

The cumulative results of the disease shows 63.33 % with soil free trunk of the neem tree and 32.66% of the trees with soil coated trunks showed symptoms of the disease (Fig.6).

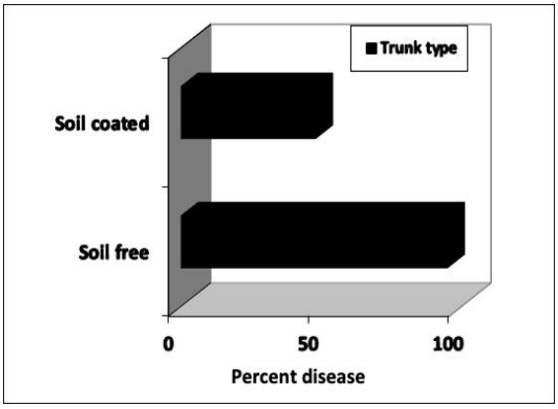

Fig. 6: Percent disease in different tree trunk type

According to the disease damage and recovery percentages at study location wise recorded, i.e., the abundant disease damage was recorded from Narva followed by Kondapur. Whereas the third position have documented from Kosgi, but the recovery rate is multiplies. The recovery percentage results however shows high recovery rate in Kosi from disease followed by Dhanwada,

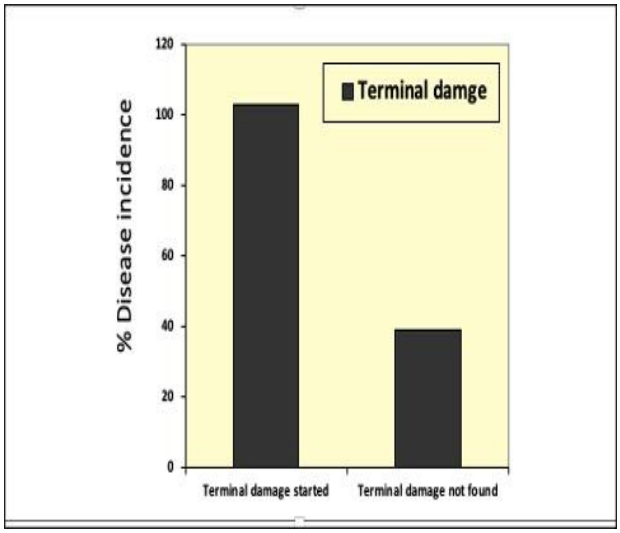

Fig.7: Observed Terminal damage and healthy buds

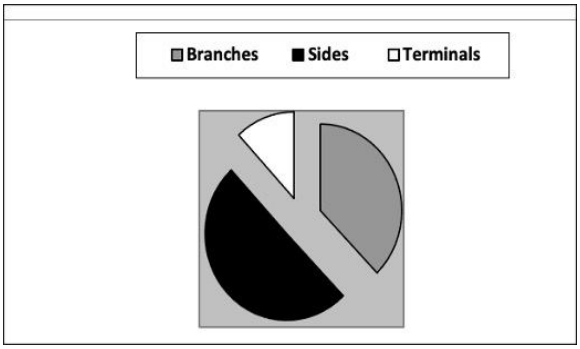

Fig.8: Percentage of Disease damages to the branches, sides and terminals

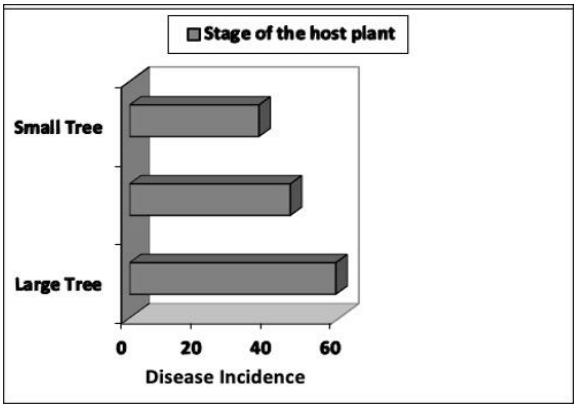

Fig. 9: Disease incidence at different stage of the trees

Narayanapet and Krishna, Maddur. The less recovery rate found at Marikal, Narva, Utkoor, Kondapur and Rampur locations. The speculate at Makthal location recovery rate and damage rate were equally recorded. (Fig.5).

A percentage of 68.6 terminal damage has been recorded, whereas in 27.33 % terminal damage

was not found (Fig.7). The recovery percentages of branches were- 36 %, side branches 48%, and terminal buds 11.33 % (Fig.8). The disease incidence documented according to the age and size of the trees were classified into three major categories like large trees (old aged), medium trees (middle aged), small trees (young aged) and disease incidence percentages of 39.33%, 30.66%, 25.33%, respectively were recorded (Fig.9).

Neem Dieback disease is spreading in an alarming manner; the fact that the illness is seed carried just adds to how widely it is dispersed. Many *Phomopsis* species are known to produce seeds, and seeds are dispersed by crop plants and tree species. Several *Phomopsis* species are widely known for causing seed rot and seed decay in different agricultural plants. *P. azadirachtae*, the pathogenic fungus that causes neem dieback disease , is found to be both seed borne and seed transmitted (Prasad *et al.* 2006). Knowing the neem seed mycoflora and seed-borne pathogens is important since neem seeds are only viable for a brief period of time. Due to its damaging effects on neem trees, the discovery of *P. azadirachtae* in neem seed is very important. Conventional procedures needed at least 15 to 21 days to isolate and identify the disease-causing pathogen. It is necessary to use sensitive techniques that can identify even extremely small amounts of pathogen propagules. Nucleic acid probes are helpful molecular diagnostic tools (Girish and Bhat, 2008). Almost everywhere where neem trees are found the disease occurs, as discovered during the survey. During the study, it was discovered that the disease incidence in the majority of the examined locations was 100% and that the severity of the disease was often as high as 100%. Neem-based insecticides are utilized in agriculture and neem cake is also used as organic manure since they are efficient, safe, biodegradable, and inexpensive. Additionally, it was discovered throughout the study that the illness attacked trees of all ages and sizes, and its severity was unaffected by climatic factors, demonstrating a high disease incidence in all agroclimatic zones. *P. azadirachtae*, the pathogenic fungus that causes die back disease on neem, is found to be both seed-borne and seed transmitted (Rajgopal *et al*., 2000), furthering the disease's broad nature. In the present study, methods like the regular one-year observations were employed to analyze the incidence of illness, which would aid in ongoing monitoring of each individual tree and its efficient management.

CONCLUSION

The present result gives awareness of the disease incidence, severity, damage and recovery in the particular present study area. Since the present selected location is at the border of Karnataka state, it is probable that the disease has spread from the Karnataka to Telangana state districts. But ultimately the total recovery has been recorded. Further, PCR and other molecular studies are required for the better documentation.

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