J. Indian bot. Soc. (2024)

e-ISSN:2455-7218, ISSN:0019 - 4468

https://doi: 10.61289/jibs2024.11.15.1139

www.indianbotsoc.org

RESEARCH ARTICLE

Evaluation of *neem* die-back damage and recovery from Palamuru University campus, Mahabubnagar, Telangana state, India

P Shivakumar Singh[1*], H. C. Shrishail[2]

Abstract

According to the current report, the Palamuru University campus in Mahabubnagar, Telangana State, India, has experienced both recovery and harm as a result of the *Azadiracta indica* dieback disease. In Telangana State, the die back illness has causes immense harm. The Palamuru campus was chosen as the area of study for the current documents. 149 sample trees in all were chosen, and they underwent periodical assessment for a year. According to an analysis of 149 trees, there were 59 large trees, 46 medium-sized trees, and 37 little trees. Where the recovery rate observed by spraying the formulations prepared using the bark extract of neem, oil of *Alium sitivam* and neem seed oil mixture under regular visits and observations, it is resulted that there is mediated recovery at normal branches (54), highly recovery at side branches and (78), and low recovery at terminal buds (17) were found. The disease initial symptoms were damage to the terminal buds, which are recorded in 17 out of the 149 trees. Additionally the secondary symptoms were also found at tree trunks as the fungi growth as soil covered. The infection was discovered in the sick trees and was then isolated. The current formulation is the first and traditional based method which is provides the basic and ecofriendly control methodology to the research community. The current report is the first and novel methodological study on neem die-back disease from southern Telangana state of India.

Keywords: Neem die-back, plant disease damage and recovery, Palamuru University campus, Telangana

Introduction

The intimate tie that exists between Indian culture and the neem tree cannot be broken. Make it clear that the Meliaceae family, often known as "Indian lilac" or "Margosa," is a subfamily of the genus Mahogany. Spread is highly widespread in India. The culture and traditions of the tree's surrounds shaped its upbringing. It is significant because it is sacred in Indian culture. Over 20 million trees can be found in India. Karnataka, India's third-most populous state, has 5.5% of the country's trees (Bahuguna 1997). Currently, neem trees exhibit higher stress tolerance when compared to other species and other criteria, including a wide range of climatic conditions, topographic adaptation, and other considerations (Chari, 1996). Here, the tree is depicted as a panacea. Neem tree parts of all kinds are used in India. for flexible pharmaceuticals. In Ayurvedic medicine, it is frequently employed. Along with strong wood, non-wood objects including flowers, fruits, nuts, oil cakes, leaves, bark, and gum are precious (Sateesh, 1998). The seeds yield 40% of the deep yellow fatty oil. usually used "Margosa Oil," which has healing properties. uses it to treat chronic skin diseases, rheumatoid arthritis, leprosy, sprains, and ulcers (Anonymous, 1992). The tree's seed cake works well as fertiliser and is easily accessible. There are 195 species of neem, and they all exhibit features of an insecticide and pesticide. As a result, the tree is also referred to locally as "Village Pharmacy," "Doctor Tree," "Wonder Tree of India," or "The Bitter Gem."

[1*]Post Doctoral Researcher, Department of Applied Botany, Kuvempu University, Shnakra Gatta, Shimogga, Karnakata, India.

[1*]Assistant Professor, Department of Botany, Palamuru University, Mahabubnagar, Telangana, India.

[2]Assistant Professor, Department of Applied Botany, Kuvempu University, Shanakra Ghatta, Shimoga, Karnakata, India.

***Corresponding Author:** P Shivakumar Singh, Post Doctoral Researcher, Department of Applied Botany, Kuvempu University, Shnakra Gatta, Shimogga, Karnakata, India and Assistant Professor, Department of Botany, Palamuru University, Mahabubnagar, Telangana, India., E-Mail: drpsksingh1@gmail.com

How to cite this article: Singh, P. S., Shrishail, H. C. 2024. Evaluation of *neem* die-back damage and recovery from Palamuru University campus, Mahabubnagar, Telangana state, India. J. Indian bot. Soc., Doi: 10.61289/jibs2024.11.15.1139

Source of support: Nil

Conflict of interest: None.

Received: 08/08/2023 **Accepted:** 15/11/2023 **Published online**: 05/01/2024

Despite being so well renowned for its biological diversity and antibacterial properties, fungal diseases affect Neem trees Shankara Bhat (1998) and Tewari (1992). The cause of the die-back of neem is *Phomopsis azadirachtae.* The fungus causes pain to the leaves, twigs, and inflorescence of all tree types, regardless of their age, size, or height. The availability of a very valuable source of botanical pesticide, the seed, has been compromised since persistently infected plants have been found to almost always experience a 100% loss of fruit output (Shankara B 1998). The illness is spreading within the three motnhs, it needs to very much quiqly identify the preliminary symptoms and removel of terminal twigs can control the spreading. In the past, there have been reports of fungi in the Mysore region (Sateesh 1998).

At the study area, in the recent years have seen a decline in number of neem trees were infected due to fungal illnesses, that attack the trees symptms and spreads from states to the borders of the neighbiour states district to districts. The location of the study area is the border of the inter state of India.

The current work undertaken for the documentation of dieback diseases sevearity and its recovery assessment from Mahabubnagar location of Telangana state, it is a first report from south Telangana and border distrct of Karnataka state, India.

Materials and methods

Disease survey

A disease survey of the neem die-back illness was conducted at the Palamuru university campus. Regular field trips were conducted in the study area (Palamuru University campus Figure 1) during the period from June 2021 to Nov 2022. A total of 150 neem trees were observed, and the locations of both damaged and healthy neem trees were mapped using the Global Positioning System (Ariane S 2013). The height, age, latitude, longitude, altitude, and degree of disease of each individual tree were recorded (Widdicombe *et al.* 1999).

Study area

The study area Palamuru University campus located in Mahabubnagar district head quarter, it comes under Telangana State, India, the climatic and environment conditions of the area comes under in subtropical region, it comprises the wet season is oppressive and overcast, the dry season is mostly clear, and it is hot year round. Over the course of the year, the temperature typically varies from *61°F to 101°F* and is rarely below *55°F* or above *106°F.* Where the soil covers sand type with red soil found. The greenery exibites with subtropical semi arid small tyes of ever green forests were found.

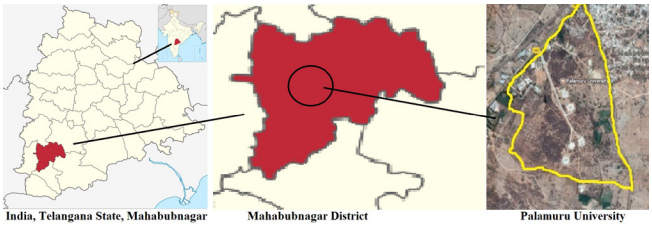


Figure 1: Study area, Palamuru University campus, Telangana State, India

Isolation of pathogen from twigs

The die back affected neem terminal buds were collected to the laboratory, and each twig had a middle transition zone and was cuts into a length of 2-3 cm. the small ieces were washes in the running water. The small pieces again cuts the transition zone area to be exposed. These segments underwent a five-minute surface sterilisation with 4% sodium hypochlorite and six to eight sterile distilled water rinses, The segments were inouculated in the agar plates. The plates were incubated for 7-8 days at 25°C with 12 hours of darkness and 12 hours of light alternately followed (Sateesh *et al.* 1997). The isolated fungi were identified using the standard procedures and manuals of the fungi idenifications Barnett H Land Hunter BB (1988) and Singh K *et al.* (1991).

Recovery treatment: The seed oil extraction was to be performed by clavenger apparatus, the bark was collected from aged tree from forests, the collected bark to be soaked in double distillued water (per 10 Lit of water 3kg small pieces of barks will be soaked), 01 ml of Allium sativam oil extracted using clavanger apparatus.

Formulation: 10 Lit. of bark extract water + 100ml of *Azadirachta indica* Seed oil + 01ml *Allium sativum* bulb oil. Mixed properly, after immediately removel of diseased twigs the formulated mixture sprayed per 20 days gap for four times, It can improves the recovery of the diseased trees. At the same time from the ground to 2 meeter of the stem should be sprayed the mixture for four time monthly once can avoide the disease spreading.

The documented information compiled, data analysised with graphical analysis has recorded.

Results and Discussion

The current information was derived from an investigation of the recovery and damage caused by the *Azadiracta indica* disease on the campus of Palamuru University at Mahabubnagar, Telangana State, India.

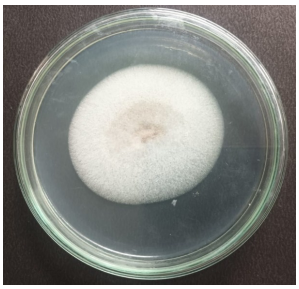


Figure 2: Isolation of dieback pathogen *P. azadirachtae*

Azadirachta indica diseased twigs collected from study locations (Figure 4), the twig parts cut into small pieces, these were surface sterilized by immersion into 1% sodium hypochlorite for 1 minute, then washed thoroughly in two changes of sterile distilled water and dried incubated for 4–5 days have shown growth of fungus mycelia. The pycnidia fabrication actually acquires 7–8 days, awhere the sporulation took bout 15 days. Cultural characteristics were recognized for all the isolates of different places (Figure 2, 3). The pathogen fungi were identified as *Phomopsis azadirachtae* by studying the culture characteristics. The foremost morphological and diagnostic characteristics of this fungus are mycelium absorbed, branched, septate, profuse colorless, becomes pale brown later. Conidiomatal pycnidia are solitary or aggregate, half immersed, pale to dark brown in color. It produces two types of conidia, alpha and beta, in cream to dark coloured slimy cirri. Alpha conidia are hyaline, fusiform straight, two-four guttulate, smooth and aseptate. Beta conidia are hyaline, filiform, eguttulate, aseptate and look like hockey sticks, besides of the pathogen fungi associated fungi like *Aspergillus fumigates,. Pancilium sps, Aspergillus niger, Aspergillus flavus, Fussarium oxysporum were* also isolated and identified (Figure 5), and their isolated parecentages also shown in Table 1.

The *Azadirachta indica* Dieback disease has had a significant negative impact on the population of neem

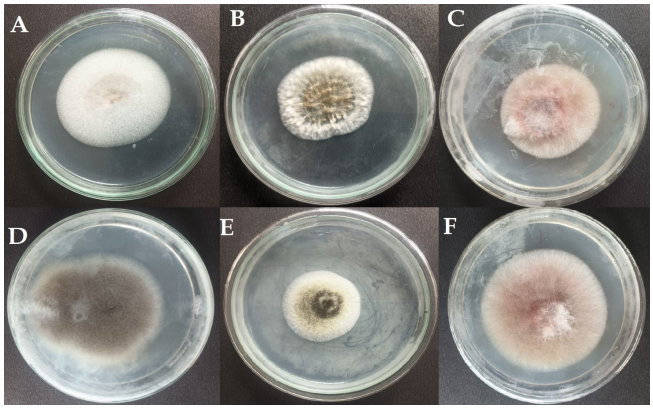


Figure 3: Isolated fungi from diseased neem tree: A. *Phomopsis azadirachtae*, B. *Aspergillus fumigates*, C. *Pancilium sps*, D. *Aspergillus niger*, E. *Aspergillus flavus*, F. *Fussarium sps*

Table 1: The Isolated fungi from the diseasesed parts and their percentage.

Sl.No.	*Isolated fungi from the diseasesed parts*	*Percentage of the isolation samples*
1	*Phomopsis azadirachtae*	98
2	*Aspergillus fumigates*	60
3	*Pancilium* sps	45
4	*Aspergillus niger*	68
5	*Aspergillus flavus*	48
6	*Fussarium* sps	55

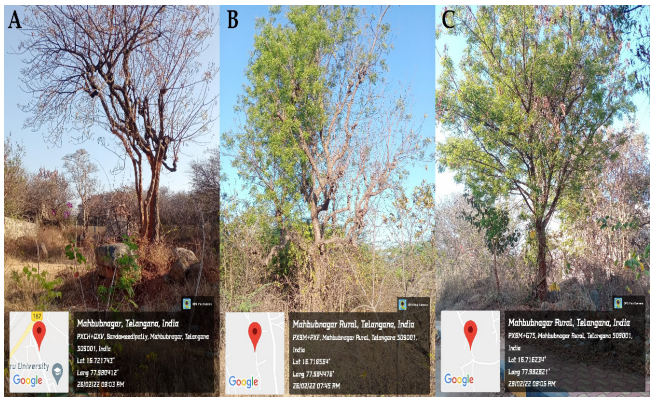


Figure 4: die-back disease sevearity and recovery stages of the *Azadirachta indica* trees., A: Total damaged tree, B: Moderate recovered tree, C: Total recovered tree.

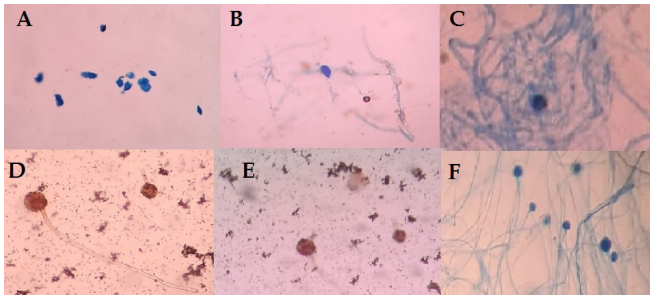


Figure5: Isolated fungi pathogens microscopic pictures: A. *Phomopsis azadirachtae* spores, B. *Aspergillus fumigates*, C. *Pancilium sps*, D. *Aspergillus niger*, E. *Aspergillus flavus*, F. *Fussarium sps*.

trees at Telangana State. The study area covers rural district, contains a variety of environmental conditions.

As a result, it was determined that the Palamuru University Campus would serve as the main focus of the current report. A minimum of 149 experimental trees were chosen and there were regularly observed in the prescribed study period, the treatment and changes were recorded. These observations results the 149 trees, there were 59 enormous trees, 46 middle-sized trees, and 37 small trees were recorded. In the study locations where the healing rate was also known through regular visits, after the spraying of the prepared mixture the early recovery of 17 terminal buds, 54 branches, and 78 side branches are seen to be recoverd from disease damage.

The initial symptoms of the disease was determined the dryness of terminal buds are the evidence of the illness. Among the 149 trees 110 trees were shown the terminal dryness. Whereas the 39 trees were shown the trunk of the tree covered with soil. The disease pathogen also isolated from the trees which were covred soil trunks.

The recovery treatment methodology and the formulation mentioned in the methodology was followed accurately. The parcentage of disease recovery was recorded in the study period i.e., the month of Jan 2021 to Aug 2022. In the study 39 trees were slowly recoverd, whereas the 110

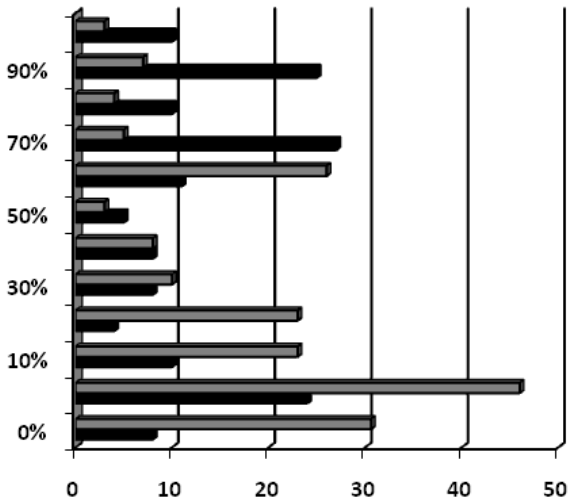
trees were recoverd quiqly, the formulation sprayed to all the 149 trees at the study region.

The natural recovery happens slowly it seems that the 39 tree were recoverd by naturally, there was no effect of the formulation on the 39 trres. The rest of the 110 trees were effectively inhibits the pathogen and recover the trees successfully. The natural observation and evaluation results may indicate the restoration of the neem trees in the state of Telangana's forests and biodiversity.

The recovery rate is dependedent on the formulation spray to the diseased trees and its parts.The recovery treatment was done by using Tthe seed oil extraction was to be performed by clavenger apparatus, the bark was collected from aged tree from forests, the collected bark to be soaked in double distillued water per 10 Lit of water 3kg small pieces of barks will be soaked), 01 ml of *Allium sativam* oil extracted using clavanger apparatus.

The mentioned oils and bark extract wer eused for the formulation preparation i.e., 10 Lit. of bark extract water + 100ml of *Azadirachta indica* Seed oil + 01ml *Allium sativum* bulb oil. Mixed properly, after immediately removel of diseased twigs the formulated mixture sprayed per 20 days gap for four times, It can improves the recovery of the diseased trees. At the same time from the ground to 2 meeter of the stem should be sprayed the mixture for four time monthly once can avoide the disease spreading.

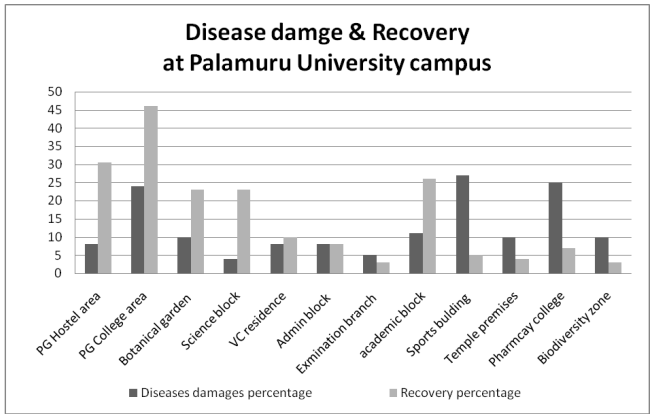
According to the illness damage and recovery results at study locations recorded, the bulk of the disease damage was found close to the locations of the sports buildings. The third position, however, has evidence from the vicinity of a pharmacy hostel, but the recovery rate is multiplied. The post-graduate college region has demonstrated a high rate of disease recovery, followed by the biodiversity zone, according to the recovery percentage data. Temple locations have a lower rate of recovery. Both the recovery rate and damage rate for the examination branch location were noted. (graph: 1)



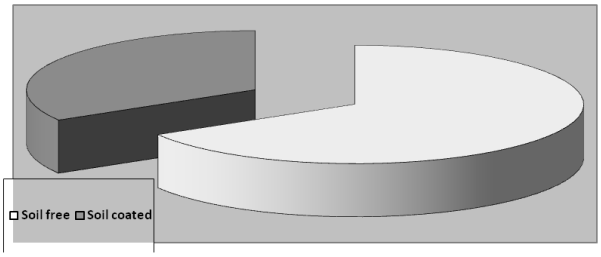
Graph 1: Diseases damages percentage & recovery percentages at Palamuru University campus

The disease's overall results show that 63.33% of cases had neem tree trunk symptoms that were not caused by dirt. Whereas, on 32.66% of the trees, the diseased plants' trunks had soil-coated symptoms (graph: 2).

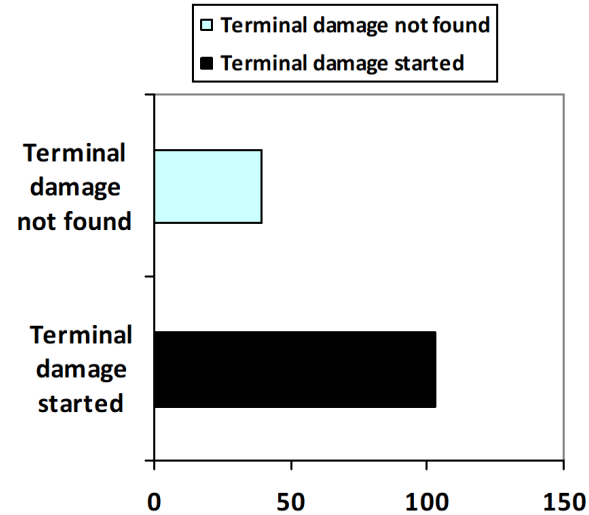
There has been 68.6% terminal damage as a percentage. Despite the fact that the 27.33% was noted to have terminal damage, it has not been found (graph: 3). The calculated rates of recovery for terminal buds (11.33%), side branches (48%), and branches (36% each) (graph: 4). According to the age and size of the trees, the percentages of disease



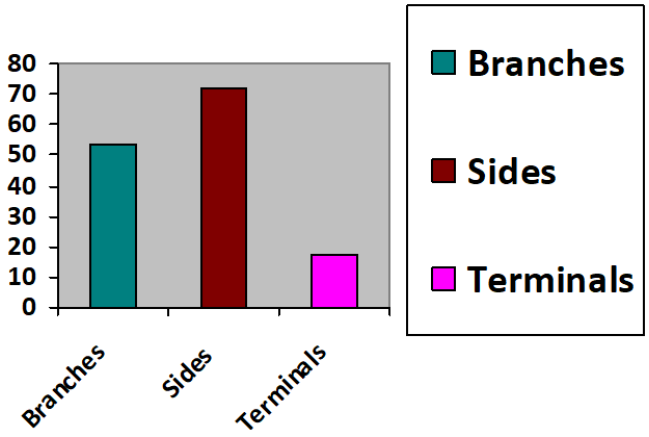
Graph 2: Diseases damages percentage & Recovery percentages at Palamuru University campus



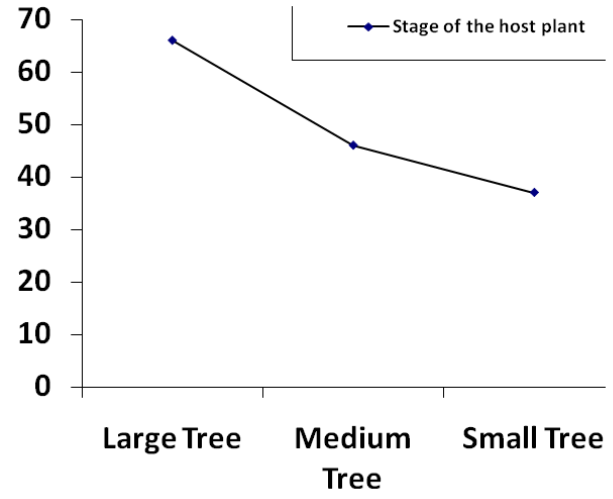
Graph 3: Diseased tree trunk type of infection with soil free and soil coated at Palamuru University campus



Graph 4: Diseased tree terminal damage found and terminal damage not found at Palamuru University campus



Graph 5: Diseased tree symptoms appeared at branches, side twig and terminal at Palamuru University campus



Graph 6: Diseased tree types at Palamuru University campus

incidence for large trees (old age), medium trees (middle age), and small trees (young age), respectively, were recorded to be 39.33%, 30.66%, and 25.33%. (graph: 5, 6).

Researchers have discovered that *Phomopsis azadirachtae,* the pathogenic fungus that causes neem dieback disease (Bhat et al. 1998), is both seed borne and seed transmitted (Sateesh et al. 1997). Neem seeds are only viable for a short time, so it's important to know the mycoflora and pathogens that are present in neem seeds. It is significant that *Phomopsis azadirachtae* was found in neem seed because of its negative effects on neem trees. To isolate and identify the disease-causing pathogen, conventional approaches need at least 15 to 21 days.

Use of advanced analysis that can detect even minute quantities of disease propagules is required. A valuable molecular diagnostic tool is a nucleic acid probe (Girish, 2008). The frightening rate at which the neem dieback disease is dispersing is made more worse by the fact that the disease is propagated by seed. There are numerous Phomopsis species that are known to generate seeds, and crop plants and tree species spread the seeds. There is widespread knowledge that some Phomopsis species can cause seed rot and seed decay in several agricultural plants.

During the survey, it was determined that the disease is almost universally present wherever neem trees are located. During the investigation, it was found that the disease incidence in most of the areas looked at was 100% and that the disease severity was frequently as high as 100%. Since they are effective, safe, biodegradable, and reasonably priced, neem-based pesticides are employed in agriculture, and neem cake is also used as organic manure. The study also showed that the disease had a high occurrence in all agroclimatic zones and that it impacted trees of all ages and sizes, with no effect on the severity of the disease from climatic conditions.

The fact that *P. azadirachtae,* the pathogenic fungus that causes die back disease on neem (Bhat et al. 1998), is both seed-borne and seed transmitted (Sateesh et al., 1997) only adds to the disease's widespread nature. Methods like the regular one-year observations were used in the current study to examine the incidence of sickness, which would help with ongoing monitoring of each individual tree and its effective management.

According to Sateesh, Bhat, and Devaki the die-back of neem is caused by *Phomopsis azadirachtae*, The neem die-back was reported using the young trees of Dehra Dun, North India (Khan, 1992), where in the current report all the age trees were cocnsiders for the research. The pathogen of the neem die-back (*Phomopsis azadirachtae)* was first identified and published by Sateesh et al (Sateesh, 1997) from Karnataka state, while in the current report the dieback recovery formulation was standardized from Telangana state.

Commonly the fruit rot, inflorescence blight, and twig blight are the responsible of slight sickness symptoms of neem tree Shankara Bhat, (1998) where the dieback disease has shown the most severe and fatal sickness infections to the regardless of their age, size, height and it spreads the year-round, specifically the symptoms exposed in the month of August to December. In the present observation the initial symptoms were recorded in the rainy to early winter session (Figure 2) this report results similar to the previous report of Sateesh, (1998).

The current report reveals the fruit yield also reduced in the dieback disease caused trees, this statement also supported by the past report of the Sateesh (1999), and the disease spread methodology via conidia, which are carried by insects, raindrops and the infection propagated through seeds were reported by Sateesh (1999) from Karnataka state. Parvathi Chary (2011) studied the comprehensive study on characterization of elite Neem chemotypes through mycofloral, tissue-cultural, ecomorphological and molecular analyses using azadirachtin-a as a biomarker. Elham S Dawood, (2015) reported on leaf extract of neem (*Azadirachta*

indica) against shoot dieback disease of two species of *Ficus* and controlled, but in thie report the treeitself under illness. Rahil Ghasemi-Sardareh and Hamid Mohammadi (2020) worked on pathogenicity and its spreading results were similar to the current report. Nasir et al., (2011) contributed on shisham dieback using neem (*Azadirachta indica*) products on seedling growth of shisham dieback pathogens and inhibited successfully. Ibrahim Yakubu, Genotype (2008) were worked on environment interactions of *Azadirachta indica* effectively. Whereas Satya RS et al., (2017) were worked used plant extracts controlled the dieback disease of Tea *Camellia sinensis* Caused by *Fusarium spp.* It also supports to the present results with dieback pathogens. P. Shivakumar singh and Shrishail HC (2023) recentlty reported the assessment of *Azadirachta indica* Die-back disease from Telangana State which was near to the current study area.

Conclusion

The latest findings can enhance the understanding of disease recurrence, severity, damage, and recovery on a specific campus of Palamuru University. Biodiversity is plentiful in the current location of choice. Neem trees are thriving in great numbers. Only 149 trees from each location were chosen from the trees overal, and the assessment was noted. it is resulted that there is mediated recovery at normal branches (54), highly recovery at side branches and (78), and low recovery at terminal buds (17) were found. The disease initial symptoms were damage to the terminal buds, which are recorded in 17 out of the 149 trees. Additionally the secondary symptoms were also found at tree trunks as the fungi growth as soil covered. The infection was discovered in the sick trees and was then isolated. The formulation which is used in the study is the first and traditional based method, which is provides the basic and ecofriendly control methodology to the research community. The current report is the first and novel methodological study on neem die-back disease from southern Telangana state of India.

There are numerous abiotic and biotic elements that have an impact on the disease's spread, damage, and for natural recovery. The current report also prepared the neem tree oil, crude extract formula and sprayed and assessed the recovery parcentages with ecofriendly. The novelty of the present report reveals the naturally the dieback diseased trees were recovered with slowly itself., on the basis of this concept the seed oil and bark extracts were mixed and formatted, sprayed. The current study can serve as wonderful knowledge for forthcoming research researchers. The formulation can essily utilized for the control of the dieback disease in the worldwide

Acknowledgments

The Vice Chancellor and Registrar of Kuvempu University, Shankara Ghatta, Shimogga, Karnataka State, are to be thanked for the chance to do post-doctoral research as well as for the facilities providing and moral support. The author are also grateful to the facilities and research support provided by the Vice Chancellor and Registrar of the Palamuru University, Mahabubnagar, Telangana state, India.

References

Anonymous (1992). *Neem – A tree for solving global problems*. National Academy Press, Washington. D.C. U.S.A.

Ariane S and Werner P (2003). Tree protection legislation in European cities Urban Forestry & Urban Greening, 2(2): 115-124.

Bahuguna VK (1997). Silviculture and management practices for cultivation of *Azadirachta indica* (Neem). *Indian For.* **123**: 379-386.

Barnett HL and Hunter BB **(1988).** Illustrated genera of imperfect fungi 4th Edition. St. Paul, MN, AP Press. PP: 218.

Chari MS (1996). Neem and transfer of technology. In: *Neem and Environment* (Vol. I), (Singh RP, Chari MS, Raheja K, *et al.* eds.). Oxford and IBH publishing Co. Pvt. Ltd. NewDelhi, India.

Elham SD (2015). Efficacy Of Leaf Extract Of Neem (*Azadirachta indica*) Against Shoot Dieback Disease Of Two Species Of Ficus In Atbara Town-Sudan. *Int J Sci. Tech.* **09(4):**77-83.

Girish K and Shankara BS (2008). *Phomopsis azadirachtae* – The Die-Back of Neem Pathogen. *Electronic J Bio.* **4(3):**112-119.

Ibrahim Yakubu, Genotype (2008). Environment interactions: survival performance of six internationally sourced provenances of *Azadirachta indica* A. Juss in a degraded land in Kano, Nigeria. The Environmentalist. **28:** 376-378.

Khan SN (1992). Record of *Phomopsis* twig blight in*Azadirachta indica* from Dehra Dun (unpublished). In: *Monograph of Neem* (Azadirachta indica *A. Juss*) (Tewari, D.N., Author). International Book Distributors. Dehra Dun, India.

Nasir A, Mumtaz AP, Abdul ML, Daolong D and Shahjahan R (2011). Effect of neem (*Azadirachta indica*) products on seedling growth of shisham dieback. *Afr. J. Microbio. Res.* **5:**4937-4945.

Singh PS and Shrishail HC (2023). Assessment of *Azadirachta indica* Die-back disease recovery and damage from Narayanapet District, Telangana State, *J. Mycopathol. Res.* **61(1):** 103-108.

Parvathi Chary (2011) studied the comprehensive study on characterization of elite Neem chemotypes through mycofloral, tissue-cultural, ecomorphological and molecular analyses using azadirachtin-a as a biomarker. *Physiol. Mol. Biol. Plants,* **17(1):** 49–64.

Rahil Ghasemi-Sardareh and Hamid Mohammadi (2020). Characterization and pathogenicity of fungal trunk pathogens associated with declining of neem (*Azadirachta indica* A. Juss) trees in Iran. *J. Plant Pathology.* **102**:1159 – 1171.

Sateesh MK (1998). *Microbiological investigations on die-back disease of neem (*Azadirachta indica *A.Juss.*). Ph.D. thesis. University of Mysore. Mysore. India.

Sateesh MK and Shankara Bhat S (1999). Detection of seed–borne *Phomopsis azadirachtae* and its transmission in *Azadirachta indica* (Neem). *Seed Sci Technol*, **27:** 753-759.

Sateesh MK, Shankara Bhat S and Devaki NS (1997). *Phomopsis azadirachtae* sp. nov. from India. *Mycotaxon,* **65:** 517-520.

Satya Ranjan Sarmah, Baruah PK and Suresh CD (2017). Practical Utilization of Botanical Extracts and Microbial in Controlling Dieback Disease of Tea *Camellia sinensis* (L) O. Kuntze Caused by *Fusarium, J. Tea Science, 7.*

Shankara Bhat S, Sateesh MK and Devaki NS (1998). A new

destructive disease of neem (*Azadirachta indica*) incited by *Phomopsis azadirachtae*. *Curr Sci,* **74:** 17-19.

Singh K, Frishvad J C, Thrane U and Mathur SB **(1991).** An llustrated manual on identification of some seed borne *Aspergilli, Fusaria* and *Penicillia* and their *mycotoxins.* Danish Government Institute of Seed Pathology for Developing Countries Pyvange Alle 78 DK- 2900.

Tewari DN **(1992).** *Monograph of neem* (Azadirachta indica *A. Juss.*). International Book Distributors. Dehra Dun. India.

Widdicombe RC and Carlisle B (1999). Geographic information and global positioning systems for tree management. *J Arboricul* 25(3):175–178.