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ANTIFUNGAL ACTIVITY OF PLANT EXTRACTS AGAINST TOMATO EARLY BLIGHT PATHOGEN (*Curvularia lunata*)

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ABSTRACT

The study was conducted to identify isolated pathogenic fungus from infected tomato plants and to test the effects of plant extracts. The synthetic fungicides (mancozeb and SA) used in this study were positive control. The isolated fungus was identified and characterized through morphological and molecular techniques based on the sequencing of internal transcribed spacer (ITS) region of 18S rDNA. The isolated fungus was identified as *Curvularia sp.* based on the observed morphological features. The obtained ITS sequencing showed above 99.81% similarity with *Curvularia lunata* in the NCBI database. The sequence of the fungus was deposited in NCBI GenBank under the accession number: ITS, SUB15802615 *Curvularia* PX631824. In vitro potency of neem leaf, bitter leaf and scent leaf on fungus causing early blight disease (*Curvularia lunata*) was conducted. Exposure of *Curvularia lunata* to different concentrations of mancozeb, and salicylic acid were effective in controlling the disease. Therefore, using of the plant extracts appeared to be effective and safe to the environment. Exposure of the pathogen to the leaf extracts significantly suppressed the mycelial growth of the tested pathogen. Analysis of Variance revealed significant differences at $p<0.05$ in the treatments against the fungal isolate. At concentrations of 1.5%, 3% and 5%, the mean percentage growth inhibitions were 86.42%, 90.12% and 91.36% and not significantly difference ($P>0.05$) compared to the control. Analysis of Variance revealed significant difference ($P<0.05$) in scent leaf n-hexane extract at 1.5% concentration and bitter leaf n-hexane extract at 5% concentration with the lowest mean percentage growth inhibition of 29.22% and 23.46% compared to the control. The results also indicated the potential of plant extracts as a better alternative to synthetic chemicals due to it being less hazardous to environment as well as human life.

1. INTRODUCTION

Early blight (EB) is a destructive fungal disease of tomato and potato plants (Adhikari *et al.*, 2017 and Sallam *et al.*, 2012). The symptoms of early blight disease first develop on the lower leaves as the plants get older. The symptoms appear as small, dark, necrotic lesions which typically have target-like concentric circles and are often surrounded by a yellowing zone. The pathogen releases enzymes and toxins that degrade and destroy the host cells, thereby making nutrients accessible to the pathogen (Rotem, 1994). Under favorable conditions, the disease spreads over all parts of the plant including the

However, the first report of the early blight disease caused by *Curvularia lunata* was in Pakistan in 2016 (HebaAlla *et al.,* 2021). It belongs to the family Dothideomycetes, is filamentous, dematiaceous, pale black or brown pathogenic fungi with cylindrical and slightly curved conidia which are responsible for several diseases of plants, animals and humans (Odyuo *et al*., 2018). This pathogen colonizes the soil and vegetation and spread through airborne spores. They are both phytopathogenic and zoopathogenic (Wilhelmus and Jones, 2001). Most species of *Curvularia* are facultative pathogens of plants and cereals mainly in tropical and subtropical areas. It causes fruit rot disease in tomato plants which is characterized by watery soaked lesions on tomato fruit which later change into thin brown to black in colour (Iftikhar *et al.,* 2016). It causes the most devastating disease and significant losses in both quantity and quality of fruit yield (Tomazoni *et al.* 2016; Perveen *et al*. 2019). *Curvularia lunata* has also been identified as a dominant pathogen on sorghum and millet seeds collected in Korea, potentially serving as a primary source of infection (Yago *et al*., 2011). It is responsible for not only grain mold in sorghum (Tarekegn *et al.*, 2006; Sharma *et al.*, 2010) but also leaf blight in other crops such as pearl barley (Dai *et al.*, 2019) and leaf spot in cotton (Shirsath *et al*., 2018).

Control of early blight disease has been effected primarily by the application of chemical fungicides (Jones *et al.*, 1991). Fungicide treatments are the most effective and efficient way of controlling early blight disease. There were several fungicides including captafol, mancozeb, benomyl, carbendazim, metiram, copper oxychloride + dichlofluanid and copper oxychloride + folpet have been successfully been used for the control of early blight (Babu *et al.*, 2001). Also, salicylic acid (SA) which is a chemical inducer in various plants. It is an important signal molecule that plays a critical role in plant defence against pathogen invasion (Chaturvredi & Shah, 2007). It can be clarified in the fight against plant diseases, as many properties of this chemical inducer have been demonstrated, as it has been widely used in medicine as an analgesic, antipyretic and anti-inflammatory agent, in addition to its bactericidal, fungicidal effects and as a protective property. More recently, it is used in agricultural operations as an alternative to fungicides, as a resistant catalyst (Wang *et al.,* 2007) and a growth promoter (Sharma, 2013). Some of these chemicals are not considered to be long-term remedies, due to concerns of cost, exposure risks, synthetic fungicide residues and other health and environmental hazards. So bio-fungicides that are eco-friendly and less expensive with no human health risks are preferred to control pathogenic fungi as compared to synthetic fungicides.

Plant extracts or phytoextracts play a significant role as antifungal agents (Parveen *et al.,* 2017; Haider *et al.*, 2020). Some of the plant extracts that have been studied in Nigeria with a view to managing postharvest losses of tomato include garlic (*Allium sativum*), ginger (*Zingiber officinale*), (Chuku *et al.,* 2010), *Tridax procumbens, Vernonia amygdalina, Chromolaena odorata, Azadirachta indica*, *Ocimum*

gratissimum (Ijato *et al.*, 2011). The quest for plant extracts in the control of postharvest spoilage of tomato fruits forms the perceived belief that plant products are usually broad spectrum, effective, economical and most importantly environmentally safe (Ramazani *et al.*, 2002; Chuku, 2006). Therefore, this study aimed at evaluating antifungal activity of selected plant extracts, mancozeb and salicylic acid against tomato early blight pathogen (*Curvularia lunata*).

2.0 MATERIALS AND METHODS

2.1 Collection of Samples: Leaves of tomato plants with early blight typical symptoms were collected from two areas in Ilorin town, i.e. Lasoju vegetable farm, Asa Local Government Area, Kwara State (8° 26′ 25″ N, 4° 29′ 40″ E) and Gaa-Saka (Residential area), Ilorin West Government Area, Kwara State (8° 28′ 53.62″N, 4° 30′ 13.63″E) during 2022 growing season. The diseased samples were brought to the laboratory and kept in the refrigerator at 4-6ºC for a few days

2.2 Media Preparation: The medium used for isolation of fungi was Potato Dextrose Agar (PDA) which was prepared according to the manufacturer's instruction.

2.3 Isolation of Fungi: The infected tissues of 5 mm^2 were cut from the advancing edges of the lesions and were surface sterilized with sodium hypochlorite (0.5%) for 2-3 minutes, then rinsed three times in sterilized distilled water for 3 mins and transferred directly to the PDA medium in 9 cm Petri dishes and incubated under 12h light and 12h dark at 25±1°C according to Naik *et al*. (2010). Pure cultures were maintained on PDA slants and stored in a refrigerator at 5-10°C.

2.4 Identification of Fungi: Identification was done microscopically and macroscopically. Colony characteristics such as appearance, change in medium colour and growth rate were observed. Shape of the conidia and conidiophores were taken note of. These features were confirmed and authenticated with the help of the database Nomenclature and Species Banks according to Robert *et al.,* 2005.

2.5 Preparation of Extracts: 50g of the leaf powder was weighed and dissolved in 500ml of distilled water and boiled for 30minutes, the boiled solution were filtered using whatman No.1 filter paper. Extraction was also done with 96% of ethanol and n-hexane heated at 78ºC for 18hours using 2000ml ethanol and n-hexane respectively.

2.6 Concentration of Crude Extracts: The extracts thus obtained were considered as standard (100%) stock solution and used to prepare desired 1.5%, 3% and 5% of aqueous extracts, ethanol and n-hexane extracts respectively.

2.7 Experimental Design and Analysis

The experiment was conducted in the Plant Biology Department Laboratory, Microbiology Department Laboratory of Osun State University. Isolated fungus (*Curvularia lunata*) was subjected to poisoned food technique to evaluate the efficacy of leaf extracts and mancozeb. 5ml each of the different concentrations of

of crude extracts (1.5%v/v, 3%v/v and 5%v/v) of the aqueous extracts, ethanol and n-hexane extracts and mancozeb at 50 and 75% concentrations respectively were dispensed into 9cm diameter Petri dishes after which 20ml of melted PDA medium was poured into the plate, then shaken together and allowed to solidify and replicated thrice. The plates were then inoculated at the center with equal discs (5mm in diameter) of *C. lunata* taken from 7 days old culture. Then plates were incubated at 28+2ºC until mycelial growth of pathogen appeared on the plates (Qasem and Abu-Blan, 1996). The antifungal activities of plant extracts were calculated and measured as percentage reduction of growth of pathogen comparing with control using the formula:

$$\frac{K1-K2}{K1} \times 100$$

Where K1-growth of the pathogen in control

K2-growth of the pathogen with treatment

T1 -Neem leaf aqueous extract T2 - Bitter leaf aqueous extract

T3 -Scent leaf aqueous extract T4 - Neem ethanol extract

T5 -Bitter leaf ethanol extract T6 - Scent leaf ethanol leaf

T7- Neem leaf n-hexane extract T8 - Bitter leaf n-hexane extract

T9- Scent leaf n-hexane extract T10 - Mancozeb at 50% concentration

T11 -Mancozeb at 75% concentration T12 - water only (CTR)

The data collected were subjected to one-way Analysis of Variance (ANOVA), the means were separated using the Duncan multiple range test (DMRT) at a 5% significance level using SPSS 20.

3.0 RESULTS

3.1 Morphological and molecular identification of the fungus

Fungus isolated from tomato leaf samples was identified to be *Curvularia sp* as shown in figure 1. It appeared black, brownish black, dark grayish, olivaceous black, pinkish red, light grey, and creamish white. Some isolates have circular or irregular growth margins, while some have rough and others with smooth margins. The conidia is separate and borne singly on the simple conidiophores. The conidia colour vary from dark muriform, pale golden, to olivaceous brown. The conidia are septate, but the number of septa varied between isolates.

Its spores are enlogated, muriform, beaked, septate and dark coloured, while the mycelia are branched and septate.

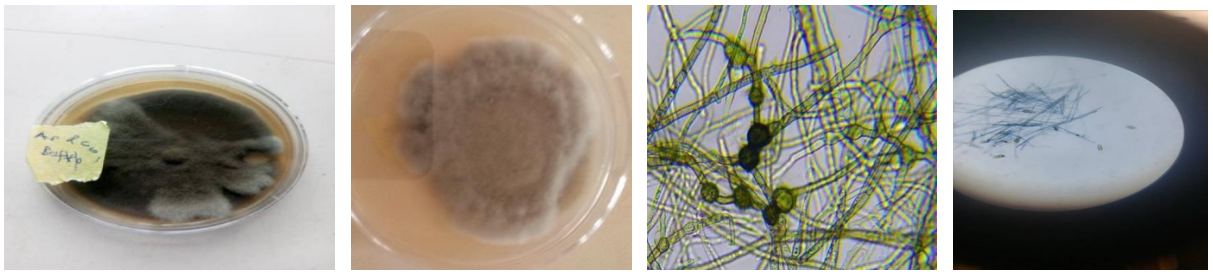


Figure 1: Plates showing Fungal colonies on PDA media (A), and microscopic view (400 X) of *C. sp.*

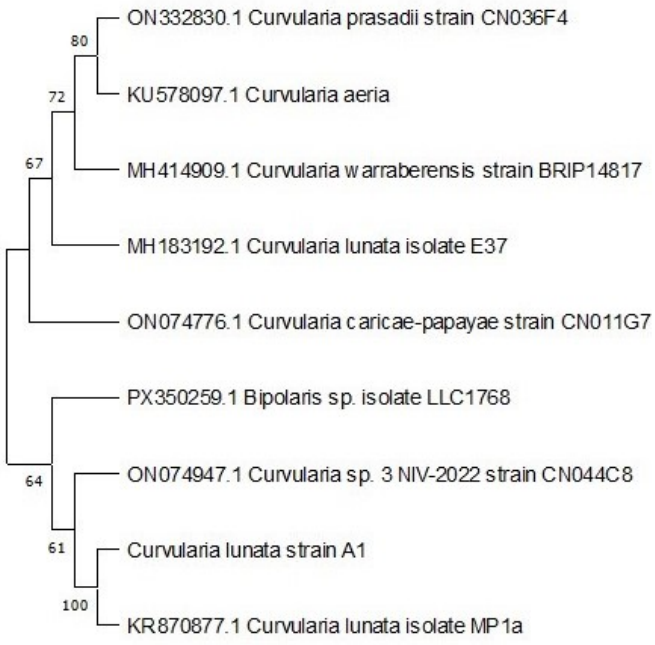


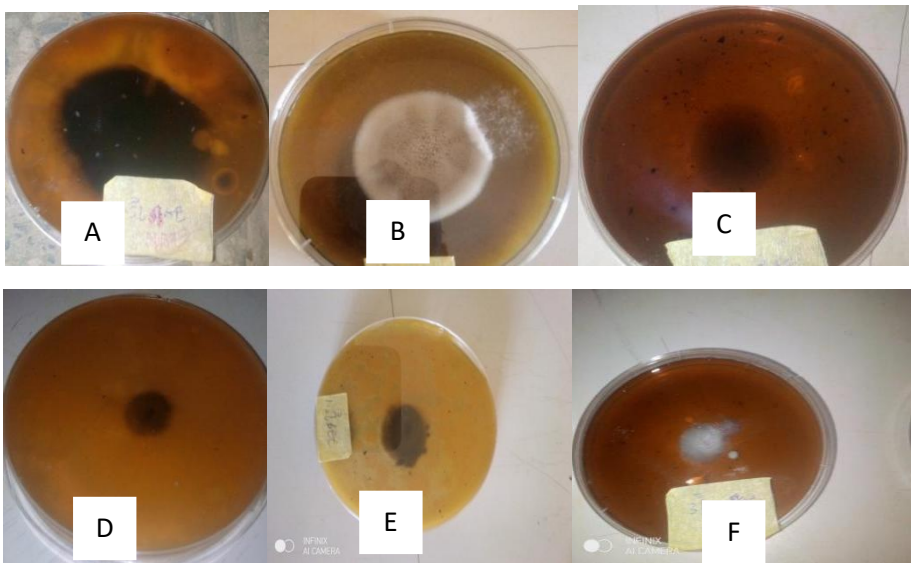
Figure 2. Maximum likelihood phylogenetic tree based ITS sequences and bootstrap support values>60 (BS) are given at the nodes (BS) on ITS sequences of rDNA of the isolated fungal strain in the present study was *Curvularia lunata* (SUB15802615 *Curvularia* PX631824) aligned with closely related sequences accessed from the Gen Bank. The marker reflects the relative phylogenetic distance measurement.

3.2 Effect of Plant extracts and Mancozeb on the mycelial growth of *C. lunata*

Gradient of efficacy of the three types of leaf extracts tested for antifungal efficacy against the test organism in this study was: scent leaf ethanol extract at 5%>scent leaf ethanol extract at 3%>scent leaf ethanol extract at 1.5%>bitter leaf n-hexane extract at 1.5%>neem leaf ethanol extract at 3%>neem leaf ethanol extract at 5%> neem leaf ethanol extract at 1.5%>bitter leaf ethanol extract at 1.5%>bitter leaf. n-hexane extract at 3%>bitter leaf ethanol extract at 3%>neem leaf aqueous extract at 1.5% >neem leaf aqueous extract at 3%>scent leaf n-hexane extract at 5%>neem leaf aqueous extract at 5%>scent leaf n-hexane extract at 3%>bitter leaf aqueous extract at 5%>bitter leaf aqueous extract at1.5%>scent leaf aqueous extract at 3% > scent leaf aqueous extract at 5%>neem leaf n-hexane extract at 1.5%>neem leaf n-hexane extract at 3%>neem leaf n-hexane extract at 5%>scent leaf aqueous extract at 1.5%>bitter leaf

ethanol extract at 5%>scent leaf n-hexane extract at 1.5%>bitter leaf n-hexane extract at 5% compared to the control as shown on figure 1 and 3 respectively.

Ethanolic extract of scent leaf at 1.5%, 3% and 5% concentrations had the highest mean percentage zone of inhibitions which showed no significant differences as they were maintained at 86.42%, 90.12% and 91.36% compared to the control respectively. This was followed by neem leaf ethanolic extract at 3% concentration with mean percentage zone of inhibition of 86.42% and bitter leaf n-hexane extract at 1.5% concentration with mean percentage zone of inhibition of 82.72%. This was followed by neem leaf ethanolic extract at 1.5% and 5% concentrations with mean percentage zone of inhibitions of 75.31% and 76.54% respectively. There were no significant differences in mean percentage zone of inhibition values of neem leaf, bitter leaf aqueous, bitter leaf ethanol extract at 1.5% and 3% concentrations, bitter leaf n-hexane extract at 3% concentration and scent leaf n-hexane extract at 3% and 5% concentrations with values of 67.49%, 67.08%, 62.55%, 60.08%, 69.96%, 67.90%, 68.73%, 61.73% and 63.37% respectively. Scent leaf aqueous extract at 1.5%, bitter leaf ethanolic extract at 5% and neem leaf n-hexane extract at 5% concentrations with with 47.74, 46.09 and 49.79% mean percentage zone of inhibitions which were not statistically different. The following treatments showed the lowest mean percentage zone of inhibitions; Bitter leaf n-hexane extract at 5% and scent leaf n-hexane extract at 1.5% concentrations with the mean percentage zone of inhibitions; 22.22% and 25.51% respectively. Mancozeb at 50% and 75% concentrations showed the following mean zone of inhibitions; 62.96% and 83.95% respectively. Lastly, salicylic acid (SA) has the highest mean percentage inhibition of 100% at 5, 10 and 20% concentrations.



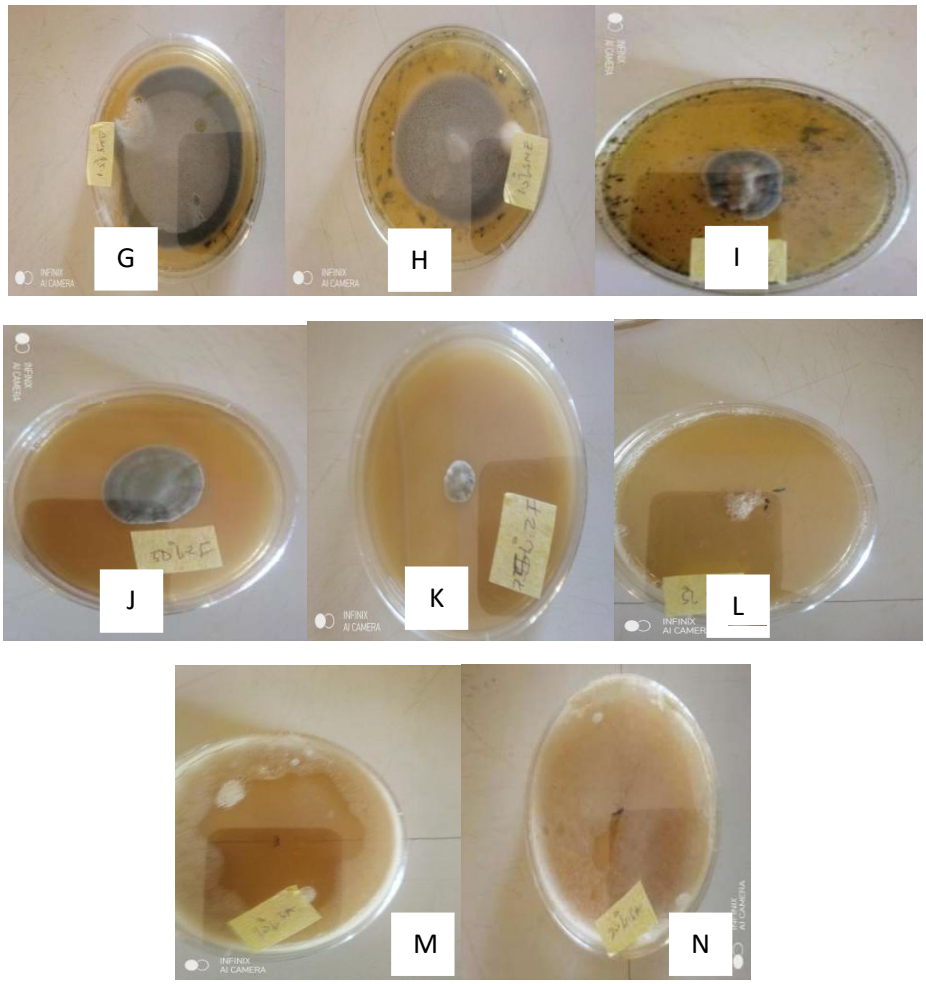


Figure 3: The effect of various leaf extracts and mancozeb at different concentrations on mycelial growth inhibition of *C. lunata* at 25±2℃ temperature.

A-Neem aqueous extract(NAE), B-Bitter leaf aqueous extract(BAE), C-scent leaf aqueous extract(SAE), D-Neem ethanol extract(NEE), E-bitter leaf ethanol extract(BEE), F-scent leaf ethanol extract(SEE), G-Neem leaf, n-hexane extract(NNE), H-Bitter leaf n-hexane extract(BNE), I-scent leaf n-hexane extract(SNE), J-50% Mancoozeb, K-75% Mancozeb, L-5% salicylic acid (SA), M-10% salicylic acid(SA) and N-20% salicylic acid (SA)

Table 1: Percentage Growth Inhibition of various plant extracts on the growth of *Curvularia lunata* at various concentrations using water, ethanol and N-hexane

S/N	TREATMENTS	1.5%	3%	5%
1	NAE	67.49±2.29d	67.08±1.48d	62.55±1.09e
2	BAE	58.02±0.71e	60.08±0.41e	58.85±1.09e
3	SAE	47.74±4.18f	55.97±0.41e	55.56±0.71e
4	NEE	75.31±0.71c	80.56±1.46b	76.54±1.89c
5	BEE	69.96±0.82d	67.90±0.71d	46.09±1.09ab

6	SEE	86.42±1.88b	90.12±0.71a	91.36±0.71a
7	NNE	52.68±0.41f	51.03±0.41f	49.79±0.41f
8	BNE	82.72±1.23b	68.73±0.41d	22.22±3.77f
9	SNE	25.51±3.93f	61.73±0.71e	63.37±1.09e
10	CTR	93.80±0.00a	93.80±0.00a	93.80±0.00a
	Pvalue	**	**	**

NS = Not significant, ** = Significant at P < 0.05 *** = Highly significant at P ≤ 0.05; Means followed by the same letter(s) in the same columns are not significantly different using ANOVA and DMRT.

NAE=Neem leaf aqueous extract; BAE=Bitter leaf aqueous extract; SAE=Scent leaf aqueous extract; NEE=Neem leaf ethanol extract; BEE=Bitter ethanol extract; SEE=Scent leaf ethanol extract; NNE=Neem leaf n-hexane extract; BNE=Bitter leaf n-heane extract and SNE=Scent leaf n-hexane extract

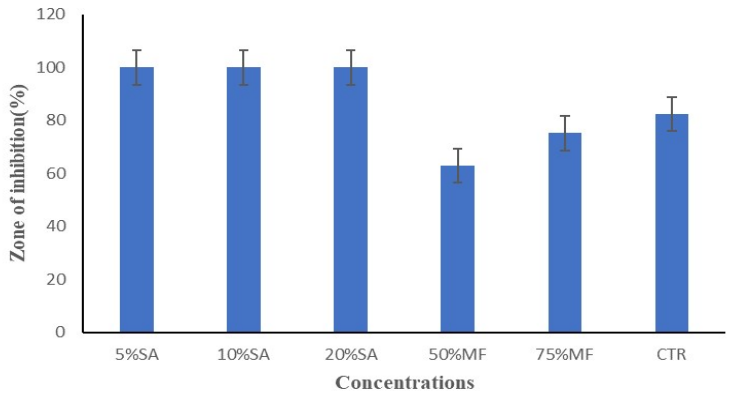


Figure 4: Percentage Growth Inhibition of Salicylic acid (SA) and Mancozeb at various concentrations on the growth of *Curvularia lunata*

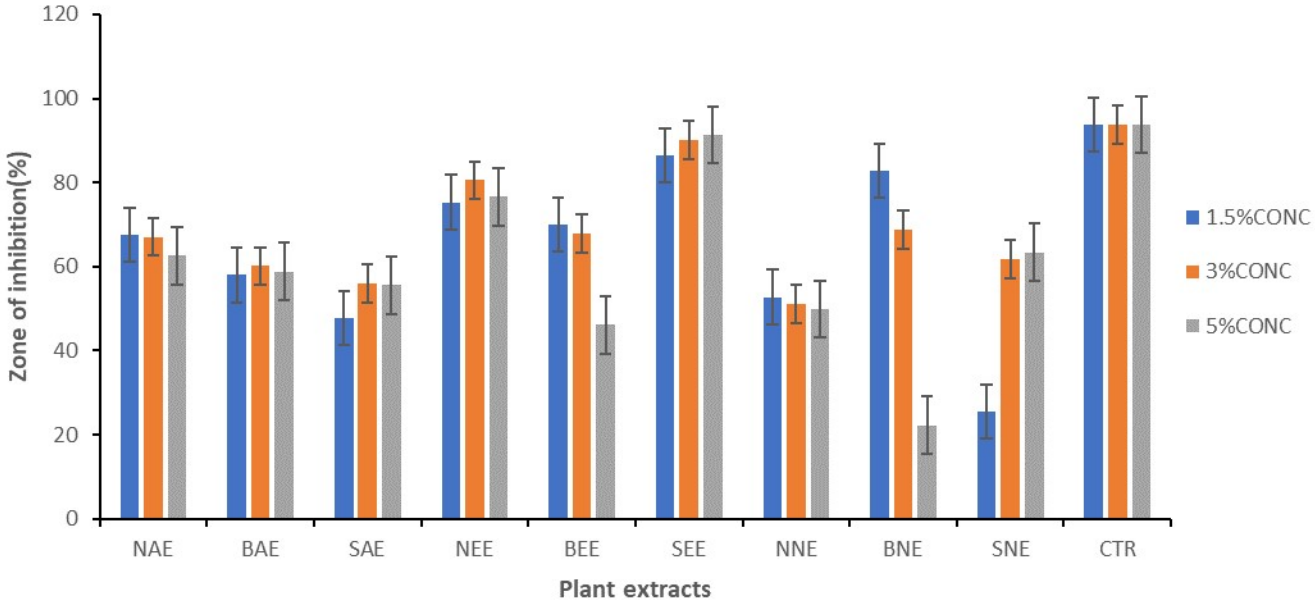


Figure 5: Percentage Growth Inhibition of Plant extracts on the growth of *Curvularia lunata* at various concentrations using water, ethanol and N-hexane

4. DISCUSSION

Many studies have shown that plant extracts effectively controlled various plant pathogens in vitro (Talibi *et al.*, 2012). Research has recently focused on using medicinal plant parts to control plant and animal diseases due to their secondary metabolites, which include alkaloids, anthraquinones, cardiac glycosides, cyanogenic glycosides, tannins, and polyphenols (Iwu, 2000). Secondary metabolites contained in the extracts of neem and bitter leaves, have been reported to possess antimicrobial and allelopathic properties which the plants containing them use as protection from diseases and damage (Saxena *et al.* 2013). The antifungal efficacy of these plant extracts were as a result of the fact that they are very rich in antioxidants such as phytochemicals and vitamins, which form the basis of their therapeutic values (Onyedikach *et. al.,* 2019 & Sarker, *et. al.,* 2020). Additionally, phytochemical components in these plants are involved in detoxifying enzymes, immune system stimulations, inflammation reductants, steroidal metabolisms and antibacterial, antifungal, antihelminthic and antiviral effectors in biological systems (Yahia *et. al.,* 2019 & Dejanovic *et. al.,* 2021). Different concentrations of each of the ethanolic leaf extracts were more effective than the different concentrations of each of the aqueous and n-hexane extracts leaf plant in this study. These observations corroborated the findings of Kagale *et al.,* 2004 that several plant extracts such as *Ocimum gratissimum, Telfairia occidentalis, Vernonia amygdalina* and *Gongronema latifolium* cultivated indigenously in South eastern Nigeria have shown the antimicrobial activity against fungal pathogens under in vitro and in vivo conditions. Plant extracts have antimicrobial activity for controlling early blight disease (Rex *et. al.*, 2019). Shrivastava and Swankar (2014) observed that different concentrations of methanolic and ethanolic neem leaf extracts have growth inhibitory effects against *Aspergillus flavus, Alternaria solan*i and *Cladosporium sp*, with the methanolic extract being the most effective against *A. flavus.* Nashwa *et al. (2012) also* reported *Ocimum basilicum, Azadirachta indica, Eucalyptus chamadulonsis, Datura stramonium, Nerium oleander,* and *Allium sativum,* caused a significant reduction in the linear growth of *A. solani* . Sanjeet *et al.* (2005) found that *A. indica* extracts provided good control of leaf spot of faba beans caused by *A. alternata* under both laboratory and field conditions. Okigbo *et al.,* 2003 also reported the fungitoxic effects of extracts of *O. gratissimum* to completely inhibit the conidia germination of Mycosphaerella fijiensis and sigatoka disease of banana. Crude extracts of *O. gratissimum* effectively exhibited antifungal activity on *Cercospora arachidicola,* which causes leaf spot on groundnut (Okri *et al.,* 2009).

Also mancozeb at 50% and 75% concentrations were effective in controlling the *C. lunata* as the mean growth percentage inhibitions were more than 50%. These observations corroborated the findings of Ghazanfar *et al.,* 2016 that the use of fungicides is considered effective approach for controlling early blight. The fungicide mancozeb has been widely

used to control early blight, reducing the effect of the disease and enhances yield of tomato (Bais *et al.,* 2019). The highest percentage inhibition of 100% was recorded on media amended with Nordox, Mancozeb, Carbendazim, A day after incubation (Honger *et al.,* 2022). The highest percentage inhibition of 100% was also recorded for salicylic acid (SA) at 5, 10 and 20% concentrations as shown on figure 3 and 4. This was supported by Wang *et al*., 2007 and Sharma, 2013 that SA is used in agricultural operations as an alternative to fungicides, as a resistant catalyst and a growth promoter.

5. CONCLUSION

It can be concluded that scent leaf ethanolic extracts are more effective against *Curvularia lunata* isolated from infected tomato leaf. Higher inhibition of fungal growth was observed at higher concentrations of the ethanolic extracts. The results also indicate the potential of plant extracts as a best alternative to synthetic chemicals due to less hazardous to environment as well as human life. These findings suggest an alternative control method to chemical control, because it has better results as they are biologically based and environmentally safe.

RECOMMENDATION

It is recommended that these extracts should be tested on other pathogens affecting both cash and food crops, as they are environmentally friendly and not harzadous compared to synthetic fungicides. It is also necessary to test the efficacy of these extracts on healthy plant grown both in the screen house and on the field.

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