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Evaluation of Anti-fungal Properties of Pawpaw and Tamarind Leaf Extracts against Fusarium Head Blight in Both Wheat and Millet

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ABSTRACT: This research on the production of antifungalwas focused on the prevention of Fusarium head blight (FHB) associated with the head wilt in both wheat and Millet, and the isolation and identification of fungi associated with Fusarium head blight in both Wheat and millet. Fusarium-damaged kernels (FDK) which are characterized as shrivelled, light, and dull greyish or pinkishof both wheat and millet., packaged in a clean polythene and transported the laboratory to preparation.500gms of the samples was disinfected in 70% ethanol for 1minute and then transferred to 10% sodium hypochlorite solution and allowed for 30mins before it was rinsed in three (3) changes of distilled water and air dried on foil paper. The samples solutions were transferred to potatoes dextrose agar (PDA) plates already prepared and we're incubated at 25°-37°C for 5days.The research work was able to confirm that Fusarium graminearum and Fusarium ventricosum are the major causes of Fusarium head blight in wheat and millet. However, secondary contaminants such as Aspergilusniger, Aspergilus flavus and Rhizopus were also present. The data collected in terms of occurrence of fungal isolates and mycelial growth per concentration of extracts for tamarind and pawpaw leaf were tabulated and analyzed using Chi-square and ANOVA using Duncan Post hoc in SPSS version 26. A p-value <.05 was considered statistically significant.

Keywords: phytochemical, extract, fungi, analysis

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INTRODUCTION

Grains are the main source of protein for Nigerians which includes maize, sorghum, millet and rice. Grainsare used in different ways in Nigeria for different purposes. Millet is one of the main grains used in Nigeria to produce kununzakifura and the process includes separating/peeling, washing, drying, milling, fermentation, molding, steaming and drying. Although not a common cereal in Nigeria, wheat is an essential food worldwide, in addition to its use as animal feed and fiber for livestock and energy production. This is the first crop in the world with a harvested area of 219 million hectares/year and an output of 760 million tons/year (average more than 10 years). In the past decade, wheat yield reached 3475 quintals/ha, an average increase of 605 quintals/ha over the previous decade (FAO, 2022). Cereal yield is affected by biotic (pests and pathogens) and abiotic stresses due to environmental conditions, stress occurrence and genetic predisposition. Among biological stresses, pathogens (i.e. fungi, viruses and bacteria) can contribute to an average overall loss of 21.5% of cereal yield worldwide (FAO, 2022). Fusarium blight (FHB) is one of the most important fungal diseases of cereal associated with various fungal species of the genus Fusarium (Summerell, 2019). Fusarium wilt causes significant yield loss in cereal because the affected grains are small, shriveled, of low mass and quality, and contaminated with mycotoxins harmful to humans and animal feed (Mcmullen et al., 2012). The main mycotoxins produced by Fusarium species are trichothecenes, zearalenones, and fumonisins, and the emerging toxins are beauvericin, enniatins, fusaproliferin and moniliformin (Ferrigo *et al.,*2016). FHB, also known as Fusarium wilt or ear scab, attributed to fundamental changes in agricultural practices, mainly reduce tillage. FHB is also known as the disease complex because more than one species of the genus Fusarium is associated with this disease in addition to the two species of the genus Microdochium (Xu, et al., 2008). The key difference between them is that Fusarium species produce more mycotoxins while Microdochium species do not produce mycotoxins. Phytochemicals are chemical components, naturally found in different parts of plants, which make many species beneficial for therapeutic uses. Indeed, leaves of papaya are known to have various health-promoting phytochemicals, as it arose from chemical analysis performed in various studies which clearly illustrated the presence of significant amounts of alkaloids, saponins, glycosides, flavonoids, phenolic compounds, enzymes, amino acids, lipids, carbohydrates, vitamins, and minerals (Alara et al., 2022).

MATERIALS AND METHODS

Sample collection and preparation

Collection and preparation of fusarium head blight wheat and millet

Fusarium-damaged kernels (FDK) which are characterized as shrivelled, light, and dull greyish or pinkish (Bushnell et al., 2003) of both wheat and millet was purchased from the Farin gada market, packaged in a clean polythene and transported the laboratory to preparation. 500gms of the samples was disinfected in 70% ethanol for 1minute and then transferred to 10% sodium hypochlorite solution and allowed for 30mins before it was rinsed in three (3) changes of distilled water and air dried on foil paper.

Collection and preparation of tamarind and pawpaw leaves

Fresh tamarind and pawpaw leaves were collected from the plants in Anguwan Rukuba area of Plateau state. It is packaged into a clean polythene bag and transported to the laboratory for processing. In the laboratory, leaves were rinsed under tap water and then by double distilled water then dried at room temperature for 15 days. It was then ground into powdered form and weighed using a digital weighing scale.

Extraction and phytochemical analysis of pawpaw and tamarind leaf powder

Twenty grams (20g) of dried leaves powder was soaked in chloroform for extraction in a rotary shaker for 3-5 days. The extract was then filtered through cheesecloth and the extract will be reduced to 10% of the original volume using a water bath at 40˚C finally dried as powder. The same process was used for aqueous extract preparation as described by Suresh *et al*., (2008) with slight modification. The phytochemical analysis for detection of secondary metabolites was carried out using the method of Paulsamy et al. (2014) with slight modification. Phytochemicals analyzed for included; Alkaloids, Phenolics, Flavonoids, tannins, and total saponins.

Isolation and identification of fungi associated with fusarium head blight in wheat and millet

From the dried grains, six (6) to ten seeds were aseptically placed in petri dishes containing solidified potato dextrose agar (PDA) and incubated at room temperature ($25^0C\pm3^0C$) for 5 days. Identification of isolated fungi was carried out both cultural and morphological characteristics as described by Barnet and Hunter (1999).

Frequency of occurrence of isolated fungi

The frequency of occurrence of the fungal isolates were determined by counting the number of times each fungal specie appeared in the mixed culture and it was expressed in percentage of the total number of fungi that appeared on the culture.

$$Percentage occurrence\ (\%) = \left(\frac{Fungal isolate}{total occurrence of all fungal isolates}\right) * 100$$

Invitro evaluation of pawpaw and tamarind leaf extracts against fungal isolates from wheat and millet

Each of the leaf extracts were weighed into 3 parts 0.5grams, 1 gram and 5 grams. These weighted extracts

Table 1: Cultural and morphological characteristics of isolates from wheat and millet with fusarium head blight.

Characteristics	Organisms Isolated				
	1	2	3	4	5
Conidia Shape	Oval to Kidney shape	Globose	Globose	Oval	Globose
Texture	Fluffy	Velvety	Velvety	Fluffy	Fluffy
Surface	Smooth	Smooth	Smooth	Smooth	Smooth
Colour	Yellowish white	Black	Yellowish-green	Pale orange to white	Whitish-black
Reverse colour	White-creamy	Yellowish	Pale Yellowish	White creamy	Creamy- white
Type of Mycelia	Chlamydospore	Cornidiospore	Conidiospore	Chalmydospore	Sporangiospore
Septation	Septate	Septate	Septate	Septate	Non-septate
Probable organism	*F. ventricosum*	*A. niger*	*A. flavus*	*F. graminearum*	*Rhizopus spp*

Table 2: Frequency of occurrence of fungal isolates from wheat and millet

Fungal Isolates	Percentage Frequency	
	Wheat	Millet
Fusarium graminearum	4(80%)	2(20%)
Fusarium ventricosum	3(60%)	2(20%)
Aspergilusniger	2(20%)	3(60%)
Aspergilus flavus	2(20%)	2(20%)
*Rhizopus*spp	1(10%)	1(10%)
p-value	0.476	0.765

N=5; X^2-wheat = 4.325; df = 4, X^2-millet = 3.42; df=4

were diluted to 100mls of molten PDA at 45^{0}C and mixed thoroughly for even distribution of the extract before pouring the media to 90mL petri dishes under aseptic condition and allowed to solidify. This produce a concentration of 5mg/ml, 10mg/ml and 50mg/ml for each of the leaf extracts. About 5mm of the solidified agar was cut using the cup borer and replaced with a disc of about 5mm of the fungal isolates. The plates were incubated at 25^{0}C for 7 – 10 days with mycelial growth being measured daily.

Statistical analysis

The data collected in terms of occurrence of fungal isolates and mycelial growth per concentration of extracts for tamarind and pawpaw leaf were tabulated and analyzed using Chi-square and ANOVA using Duncan Post hoc in SPSS version 26. A p-value <.05 was considered statistically significant.

RESULTS

Fungal isolates from wheat and millet with fusarium head blight

A total of 5 fungi species were isolated from the both wheat and millet with fusarium head blight. Two (2) of the five have been reported to be associated with fusarium head blight i.e. *Fusarium graminearum* and *Fusarium ventricosum.* However, other fungi organisms isolated are *Aspergilusniger, Aspergilus flavus* and *Rhizopus* spp. based on cultural and morphological characteristics in (Table 1). In terms of their percentage occurrence, *Fusarium graminearum* was observed to be the highest with a frequency of 4(80%) followed closely by *Fusarium ventricosum.* with a frequency of 3(60%) while the least was *Rhizopus* sp. with a frequency of 1(10%) in wheats. In millets, *Aspergilusniger* recorded the highest prevalence of 3(60%) while *Rhizopus* sp. was the least with 1(10%) prevalence. The *Fusarium* species were observed to show a prevalence of 2(20%) in millets. Details in (Tables 1 and 2). The variation in the prevalence of this isolates was found to be statistically not significant (p-value = 0.476; X^2 = 4.325; df = 4) in wheats and in millet (p-value = 0.765; X^2 = 3.42; df=4).

Phytochemical properties of pawpaw and tamarind leaf extracts

Six phytochemical properties were evaluated in the ethanolic extract of the two leaves; C. papaya and *T. indica*. Both showed a variety of phytochemical properties in various degrees. However, *T. indica* showed the presence of a high quantity of the various phytochemicals tested as compared to that of *C. papaya*. Three (3) of the six (6) phytochemicals were detected to be in high abundance in *T. indica* which includes; Flavonoids, Saponin and Phenolics while in *C. papaya* only two (2) were in high abundance i.e. Alkaloids and Triterpenes. The other phytochemicals were abundant in low

Table 3. Phytochemical composition of *C. papaya* and *T. indica* leaf extracts

Phytochemical properties	*Carica papaya*	*Tamarindus indica*
Alkaloids	+++	++
Flavonoids	++	+++
Saponin	+	+++
Triterpenes	+++	++
Phenolics	++	+++
Tanins	-	++

+++ Present in High quantity; ++ Present in average quantity:
+ Present in low quantity: - not present

Table 4. Effects of leaf extract of *C. papaya* on *F. graminearum* and *F. ventricosum* from Wheat and Millet

Grain	Fungi isolates	Mycelial Radial growth(mm)			P-value
		50mg/mL	10mg/mL	5mg/mL	
Wheat	*Fusarium graminearum*	10.23±1.23[a]	12.03±2.32[a]	17.85±2.19[b]	0.023
	Fusarium ventricosum	13.34±0.12[a]	13.76±1.76[a]	20.54±3.21[b]	0.035
Millet	*Fusarium graminearum*	14.32±0.46[a]	17.35±2.31[ab]	24.78±0.32[b]	0.042
	Fusarium ventricosum	13.29±1.34[a]	15.23±1.99[ab]	21.65±0.35[b]	0.012
p-values		0.076	0.098	0.123	

Mean±Std of triplicate measures of radial growth within the same row with superscripts of different alphabets are statistically significant (p-value <.05).

quantities except for tanins that was completely absent in *C. papaya*. Details in (Table 3).

Evaluation of the effect of *C. papaya* leaf extract on the mycelial growth of organisms causing fusarium head blight in wheat and millet

Table 4 shows the various radial growth of two fungi isolates that are known to cause fusarium head blight in cereals while being treated with ethanolic extract of C. papaya at varying concentrations. A common pattern observed was a decrease in radial mycelial growth with increase in concentration in both fungal isolates from both grains. The 50mg/mL concentration was observed to inhibit growth of both *F. graminearum* and *F. ventricosum* with a mean radial growth of 10.23±1.23 and 13.34±0.12 respectively and 14.32±0.46 and 13.29±1.34 in wheat and millet respectively. The 5mg/mL concentration showed the least inhibition with mean radial growth of 17.85±2.19 and 20.54±3.21 in the isolates from wheat and 24.78±0.32 and 21.65±0.35 in the isolates from millet. The inhibition rate observed in the isolates from wheat is observed to be higher than that observed in the isolates from millet in all the concentrations. In wheat, *Fusarium graminearum* was observed to be better inhibited than *Fusarium ventricosum* while reverse is the case in millet with *Fusarium ventricosum* recording the lowest radial growth (13.29±1.34) than *Fusarium graminearum* (14.32±0.46).

The variation in mean mycelial growth with respect to the concentrations recorded was found to be statistically significant (p-value < .05) for both isolates from wheat and millet per fungi isolate. While that between the isolates from wheat and millet was found to be statistically not significant (p-value >.05) for the two fungal species isolated. Details in (Table 4).

Evaluation of the effect of *T. indica* leaf extract on the mycelial growth of organisms causing fusarium head blight in wheat and millet

For T. *indica*, the three (3) concentrations were observed to be more potent in inhibiting the growth of the two fungal isolates known to cause fusarium head blight in cereals. However, like the case was with C. papaya, the highest concentration i.e. 50mg/mL dilution was observed to record the lowest radial growth 8.43±0.23 for F. graminearum and 7.54±0.12 for F. ventricosum for the isolates from wheat and 7.12±0.16 for F. graminearum and 6.09±1.24 for F. ventricosum for the isolates from millet while the 5mg/mL recorded the highest. Considering the variation in mycelial growth of the two isolates, Fusarium graminearum was observed to record the highest measure in most of the concentrations both in wheat and millet isolates. Statistically, the variations observed in terms of the concentration of the extracts, the fungal isolates and the grains were all found to be statistically not significant (p-value > .05). details in (Table 5).

DISCUSSION

Isolates associated with fusarium head blight in millet and wheat

Although, 3 of the 5 fungal isolates in this research work have not been reported to cause Fusarium head blight, they however have reported to be associated with grains

Table 5. Effects of leaf extract of T. Indica on *F. graminearum* and *F. ventricosum* from Wheat and Millet

Grain	Fungi isolates	Mycelial Radial growth(mm)			P-value
		50mg/mL	10mg/mL	5mg/mL	
Wheat	*Fusarium graminearum*	8.43±0.23[a]	9.03±1.32[a]	12.85±0.19[a]	0.234
	Fusarium ventricosum	7.54±0.12[a]	9.76±2.76[a]	11.54±1.21[a]	0.451
Millet	*Fusarium graminearum*	7.12±0.16[a]	8.05±1.31[a]	10.78±0.32[a]	0.092
	Fusarium ventricosum	6.09±1.24[a]	7.03±0.99[a]	9.58±1.35[a]	0.120
p-values		0.760	0.198	0.0673	

due to the high nutritional value in the grain seeds. In the study by Adam *et al*., (2022) in Zaria, Kaduna state, *Aspergilus* species alongside other fungal genera are basic colonizers of grains such as rice and Legumes such as groundnut, tiger etc. which is in agreement to the findings of this research work where *Aspergilus flavus, Aspergilus fumigatus* and *Rhizopus*spp alongside *Fusarium*spp were isolated in millet and wheat. The proximate composition of cereals generally has been found to be contain carbonhydrates, protein, fibre, fats among others which can favor the growth and colonization by this fungal groups especially during storage.

However, with respect to Fusarium Head Blight (FHB), both Millet and Wheat were found to infect with *Fusarium graminearum* and *Fusarium ventricosum* but of which have been reported to be associated with FHB. In the research by Tabassum, (2021) in determining the prevalence and diversity of FHB in oats, he reported the prevalence of various Fusarium species chief of which was *F. graminearum* that recorded a prevalence of 54%, 29% and 40% in the years 2016, 2017 and 2018 respectively in Manitoba. *F. ventricosum* on the other hand has been implicated with FHB only in few literatures. Khan *et al*, (2020) in their work on the fusarium head blight in wheat: contemporary status and molecular approaches reported *F. ventricosum* to also be associated with FHB. The implication of *F. ventricosum* alongside *F. graminearum* suggests an increase in virulence by the Fusarium species on cereals leading to an increase loss of grains during storage and since these fungi are toxin producing, consumption of such infected grains can lead to food intoxication alongside other complications.

Phytochemical properties of *C. papaya* and *T. indica* leaves

Most plants and plant materials have been reported to contain various degrees of phytochemicals and secondary metabolites with some phytochemical properties on pathogens. Chavez-Quintal et al., (2011) considered the antifungal activities of C. papaya leaves and seeds on Fungi organisms, they reported the presence of secondary metabolites that are antimicrobial in nature such as the abundant presences of Alkanoids, Flavonoids and Tritepenes in the ethanolic extracts of *C. papaya* leaves and seed which agrees with the findings of this research work. However, other phytochemical properties such as phenolics and tanins were also found in this work. The increase number of metabolites in this research work to that of Chavez-Quintal et al., (2011) could be associated with differences in soil composition in the study sites as well as the variety of *C. papaya* used in the research work. *T. indica* is not different, it has been reported by Gomathinayagam et al., (2017) to contain high quantities of Phytochemical properties. In their research the reported a presence of Alkaloids, Flavonoids, Tannins, Phenols, Triterpenes, Saponins and much more which have the potential for antimicrobial activities. This is similar to that reported in this research work although in their work the quantity of this metabolites was not reported but this research showed a high abundance of Flavonoids, Saponins and Phenols all of which can combine irreversibly with nucleophilic aminoacids in fungal proteins. Moreover, they inhibit proteins to form several hydrogen and ionic bonds and disturb three-dimensional structure transporters.

Antifungal effect of *C. papaya* and *T. indica* on isolates of fusarium head blight in wheat and millet

Leaf extracts of both plant showed a significant Mycelial radial growth inhibition which can be affiliated to the phytochemical compositions presented earlier and which agrees to findings of Gomathinayagam et al., (2017) that suggested that the antioxidant and antimicrobial activity of *T. indica* leaves which has been established worldwide is a product of the high nitrogen and phytochemical constituents present in the plant.The implication of concentration in the inhibitory effect of plants extract cannot be overemphasized as demonstrated by the findings of this research. The higher the concentration of the extract, the higher its impact on mycelial growth of the fungi; this can be due to the increase in phytochemicals as concentration increases leading to a higher inhibition properties..The statistically insignificant variation in mycelial growth inhibition between the isolates of wheat and that of millet could be due to the fact that same fungal species were isolated in the two cereal seeds and since both seeds are possibly a product of same soil cultivation, the isolates have same antifungal exposure hence resistance rate are probably equal. Also, the variation in mycelial inhibition with respect to the fungal specie could be associated with the fact that *F. graminearum* has been reported severally as the major cause of FHB hence its resistance to antifungal could be high while *F. ventricosum* was associated from what can be considered a secondary location of habitation. This agrees with the argument by Leach et al., (2013) that resistance to antifungal agent can be greatly influenced by the growth state of the fungal organisms as well as the source of isolations.There is evidence to suggest that leaves contain a wide range of phytochemicals, including carpaine, kaempferol 3-(2G-glucosylrutinoside), kaempferol 3-(2″-rhamnosylgalactoside), 7-rhamnoside, kaempferol 3-rhamnosyl-(1->2)-galactoside-7-rhamnoside, luteolin 7-galactosyl-(1->6)-galactoside, orientin 7-O-rhamnoside, 11-hydroperoxy-12,13-epoxy-9-octadecenoic acid, palmiticamide, and 2-hexaprenyl-6-methoxyphenol (Soib et al., 2020). Due to these potent bioactive components, the above leaf extracts can be used in the manufacture of nutritional supplements and herbal medicine formulations. There are reports that *C. papaya* leaves are used along with other herbs to cure ailments. Traditional doctors in Nigeria use it to treat diabetes, Cameroon uses it in combination with other herbs to treat malaria and other fungal infections, and Australian Aborigines use the leaves as an anti-cancer agent. It has been shown to use decoctions (Sharma et al., 2013).

Conclusion

The research work was able to confirm that *Fusarium graminearum* and *Fusarium ventricosum* are the major causes of Fusarium head blight in wheat and millet. However, secondary contaminants such as *Aspergilusniger, Aspergilus flavus* and *Rhizopus* were also present**.** Phytochemical properties of *C. papaya* and *T. indica* showed the presences of Alkaloids, Flavonoids, tannins, triterpenes and saponins which implies that leaf extracts of this plant possess antimicrobial metabolites in varying degrees and hence can be explored as alternates for microbial inhibitions of pathogens both for plants, animals and Humans. The inhibitory properties expressed by the leaf extracts of *C. papaya* and *T. indica* on isolates of FHB suggest, its potency in controlling fungal infestation of cereals/grains during storage and even during cultivation. It is also important to note that concentration plays important role in ensuring a higher inhibitory effect

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