Millet Resistance Prospects and its Growth Associated Microorganisms Control Using Weed Clearance Herbicides

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doi.org/10.64643/IJIRTV13I4-208823-459

***Abstract*—The millet resistance prospects and its growth associated microorganisms control using weed clearance herbicides was assessed using the gardens and the Laboratory of Plant Science and Biotechnology, Aliero Nigeria. Microorganisms associated with the growth and rhizosphere of the millets comprising four bacteria and four fungi were obtained. Pathogenicity of the disease causing members was conducted to validate the status, and disease incidence and severity were measured. Fresh extracts of millet leaves growing in the farms were made using the sohxlet extraction method. Concentrations of 400, 300 and 200mg/ml were used for in vitro control of the microorganisms, using agar well diffusion method. Paraquat (4.00, 5.25 and 7.25mg/ml) and glyphosate (5.25, 7.25 and 10.00mg/ml) herbicides were also obtained and used for the in vivo damping off and rust diseases control. The highest 10.00 mg/ml concentration inhibited X. campestris by 12.67 mm, X. axonopodis by 12.67 mm, A. vinelandii by 15.33 mm and R. phaseoli by 14.33 mm. There was significant difference ($p<0.05$) between control drug and the three extract concentrations. No survival was observed in damping off but rust control was slightly detected at low concentrations of both paraquat and glyphosate, with 2.83 and 2.23, respectively. Control of damping is achievable with low concentrations of weed clearance herbicides and there is need to further look at these potentials.**

***Index Terms*—Millet, Herbicides, Fungi, Bacteria, Disease control**

I. INTRODUCTION

Millets are small grains anciently grown in arid and semiarid regions of the world. They represent a staple food for many people in Asia and Africa and are abundant sources of vitamins and minerals, giving them the name Nutricereals (Jacob et al 2024). The WHO (2024) names millets as important in correcting lifestyle disorders. Regular consumption of millet fibers has been linked to improved gastrointestinal function, enhanced nutrient absorption, and reduced risk of various digestive disorders (Dayakar et al 2016). Rust, disease of pearl millet appears in later stage of crop growth usually occurs at the stage of seed development, generally after the grain-filling stage thus causing little loss in grain yield. This disease appears on the leaf as round to elliptical reddish orange pustule. On pearl millet crop, symptoms initially appear as uredinia pustules having circular and reddish-brown to reddish orange coloured uredospores. Death of the diseased leaf moves from top to bottom. At later stages of disease, telia replaces uredinia which is ovoid, black, and subepidermal. Cluster of spores (urediniospores) develop in the pustules which become responsible for spreading the rust disease among the crops (Singh et al 2021).

Millet diseases have been reported for a long time. In India and Western and Southern Africa, much research has been done and reported about downy mildew (Sclerospora graminicola Sacc (Hash et al., 1999), as being the most important pearl millet disease in Asia and Africa (Williams, 1984); Eradication involves the biological control of pathogen, crop rotation, removal and destruction of diseased plant or organ, heat and treating the diseased plant and soil with chemical (Reddy et al., 2014).

Herbicides are principally weed killers. Conditions for efficacy of herbicides in a specific crop include weather and temperature, which may be mild, temperate or tropical (Hunsche and Noga, 2012), along with humidity and plant vigor playing important

roles, and these conditions are necessary in designing herbicide application strategies (Long et al., 2013). Understanding leaf surface coverage area, leaf surface properties and herbicide chemical properties is very important for maximum success (Menendez and Bastida, 2004). Their use for pathogen control is not widely documented or investigated, making the current study an important step.

II. MATERIALS AND METHODS

The study was conducted in Aliero, the Headquarters of Aliero Local Government in Kebbi State Nigeria. Located on latitude 113 'S, 12° 44' N and longitude 36 °W, 4 °E. It is on 247.54 M above sea level (Distanceto.com, 2026). The hottest temperature is 103°F in April and coldest month of January, with an average low of 66 °F. The rainy period of the year lasts for 6.3 months, from April 13 to October 23, with a sliding 31-day rainfall of at least 0.5 inches (Weatherspak.com, 2026).

Millet Extract Production

Standard method was adopted in the preparation of extract using the sohxlet extraction method, in which 60 g of millet was poured into the thimble and extracted using 50:50 hydroalcohol, and the extract dried using the water bath at 85 ^{0}C (Zhang et al., 2014). The procedure was also according the standard laboratory practices.

Antimicrobial Activity of Herbicides and Extract In Vitro

In vitro control abilities of the two herbicides and the extract was assessed, using the disc plate method in 90 mm petri dishes, employing the procedures as enumerated by Aneja (2018) as modified. Already prepared media were poured into 90 mm petri dishes in a laminar flow and allowed to solidify. To the media, bacteria and fungi isolates were streaked and one disc each representing the different concentrations was placed at equidistant places and adequately labeled. Bacterial and fungal growth around the discs was observed and the percentage inhibition calculated. The plates were inoculated at 28 ^{0}C + 2 ^{0}C and observations started from 24 hrs. Streptomycin (25 ug/ml) and Nystatin 10ul/ml were used as standard controls for bacteria and fungi, respectively. Herbicides and extracts were tested by spraying the seedlings, using a 2 liter hand sprayer on previously inoculated seedlings. The results were observed and noted. Inoculated diseased plants were observed for the effects of the herbicides and possible recovery from the activities of the damping off and rust pathogens, to determine their disease control capabilities.

Herbicide Control of Millet Diseases In Vivo

Spray method of inoculation was employed in the inoculation of the seedlings and the application of herbicides. Before sowing, the seeds were surface sterilized with 2% sodium hypochlorite solution for 5 min, and then soaked three times in sterilized distilled water (SDW) to get off from any contamination of microorganisms (Duggar and Davis, 1989). In every three days, each pot was watered with 200 ml of tap water early in the morning. The crop was thinned down to 5 stands per pot after 3 weeks of growth. When the seedlings were six days and ten old for damping off and rust respectively, the leaves were thoroughly cleaned with SDW and the leaves were predisposed to nearly 95 percent humidity for 24 hours (Sreenivasaprasad et al., 2005).

The spore suspensions of 15 days old culture Xanthomonas campestris and Fusarium oxysporium isolate cultures with SDW and adjusting the concentration of spore suspension to be (105 spore/ml) (Takan et al., 2004). The spore suspension contained a mixture of micro and macro conidia. Two hundred (200 ml) spore suspension was prepared for each tested isolates. Thereafter, they were inoculated by spraying on leaves by using 2 L hand sprayer (Han et al., 2003). Similarly control plants were sprayed with SDW for comparison. After application of spore suspensions, the leaves of the inoculated seedlings were covered by plastic bag and were kept for six days to create conducive condition with high relative humidity near to 95% and to ensure successful penetration and establishment of the test pathogen on the leaf and tissues of seedlings (Sreenivasaprasad et al., 2005). The plastic bags were removed after six days and plants were kept under greenhouse conditions and periodical observations were made regularly for the first appearance and development of symptom on the leaves. All treatments were replicated three times. The mean minimum and maximum temperature in the greenhouse during the study period were 12 ^{0}C and 30 ^{0}C, respectively. After 21 days of inoculation plants were assessed and recorded for the percentage of disease incidence and severity of leaf infection (Getachew et al., 2014). Statistical analysis

for all data generated was performed using SPSS (ANOVA) at significance of $p < 0.5$ confidence level.

III. RESULTS AND DISCUSSION

Anti Microbial Activity of Millet Extract

The inhibitory activity of Millet extract on bacteria is presented in Table 1, where the 200 ml/l showed no inhibitory action against X. campestris. There was however a slight inhibition of X. axonopodis by 3.67 mm. The two bacteria of A. vinelandii and R. phaseoli were also not inhibited by this concentration. The second 400 ml/l concentration showed inhibition of X. campestris by 3.67 mm, the same with X. axonopodis and A. vinelandii. Inhibition of R. phaseoli was by 11.00 mm. The highest 10.00 mg/ml concentration inhibited X. campestris by 12.67 mm, X. axonopodis by 12.67 mm, A. vinelandii by 15.33 mm and R. phaseoli by 14.33 mm. There was significant difference ($p<0.05$) between control drug and the three extract concentrations. There was also significant ($p<0.05$) between the 400 ml/l concentration which performed better than the other two concentrations.

Extract inhibition of fungi revealed no activity in all fungal species by the 200 ml/l. there was also no inhibition of A. niger by 300 ml/l but the growth of R. stolonifer, C. laurentii, F. oxysporium and M. mucor was slightly inhibited by 3.67, 7.33, 7.33 and 3.67 mm respectively. The 400 ml/l inhibitory activity was also very slight on A. niger and R. stolonifer with 3.67 and 7.33 mm respectively. C. laurentii, F. oxysporium and M. mucor were inhibited by 12.00, 12.33 and 7.33 mm respectively. There was significant difference ($p<0.05$) in the activities of the extract relative to control, and there was also a better significant activity by the 400 ml/l extract concentration than the 300 and 200 ml/l.

Table 1: Antimicrobial Susceptibility of the Microorganisms to Millet Extract

	Microorganism	Control	Extract (mg/l) 200	300	400
Bacteria	X, campestris	18.00+0.20a	0.00+0.00c	3.67+0.63c	12.67+0.05b
	X. axonopodis	18.00+0.20a	3.67+0.63c	3.67+0.63c	12.67+0.05b
	A. vinelandi	17.00+0.43a	0.00+0.00c	3.67+0.63c	15.33+04ab
	R. phaseoli	18.00+0.20a	0.00+0.00c	11.00+0.05b	14.33+04ab
Fungi	A. niger	15.00+0.44a	0.00+0.00	0.00+0.00c	3.67+0.63c
	R. stolonifer	15.00+0.44a	0.00+0.00	3.67+0.63c	7.33+0.58c
	C. laurentii	17.00+0.20a	0.00+0.00	7.33+0.58c	12.00+0.43b
	F. oxysporium	16.00+0.35a	0.00+0.00	7.33+0.58c	12.33+0.05b
	M .mucor	15.00+0.44a	0.00+0.00	3.67+0.63c	7.33+0.58c

Means in a column with same subscripts are not significantly different ($P<0.05$)
Values are mean + standard error of thee replicates.

Disease Incidence and Severity

The values calculated for the two diseases of rust and damping off as they affected the two millet varieties showed in Table 2 indicated that the incidence of damping off disease was 87.3% in Bahaushe Millet, while the incidence of Rust disease was 78.6% in the same Bahaushe Millet. In Dansalka variety, the incidence was 95.3% for Damping off disease while it was 71.9% for rust. Disease severity was observed to be very high also as the Damping off in Bahaushe was severe to the extent of 100%, which lead to total mortality of the Millet seedlings. The severity of rust was also high in Bahaushe as it rose to 85.5%. Damping off of Dansalka Millet seedlings was also very severe as mortality recorded was 100%, and resulted in the death of all the seedlings. Dansalka Rust disease was observed to be 80.4% severe. Incidences of millet diseases have been reported up to 73.585 of Rust, 62.98% of Ergots, 61.2% of Blast and 26.76% of Smut were recorded by Lubadde et al. (2014).

Analysis of the significance by T-test revealed that the incidence between both Damping off and Rust in Bahaushe was significant ($p<0.05$) but there was no significance ($p<0.05$) between the Rust incidence in Bahaushe and Dansalka. The incidence between Damping off in Dansalka and Bahaushe was significant ($p<0.05$). Also the incidence between Damping off and Rust both in the Dansalka variety

were significantly different ($p<0.05$). Disease severity of Damping off and Rust, both in Bahaushe was significantly different ($p<0.05$), the same with the damping off and Rust in Dansalka. However, there was no significant difference ($p<0.05$) between damping off in Bahaushe and dansalka. There was also no significant difference ($p<0.05$) between Rust of Bahaushe and Dansalka.

Table 2: Percentage Disease Incidence and Severity in Bahaushe and Dansalka

Disease	Incidence		Severity	
	Bahaushe	Dansalka	Bahaushe	Dansalka
Damping off	87.3+0.98*	95.3+1.83**	100+3.17*	100+4.67*
Rust	78.6+2.65	71.9.+3.33	85.5+1.83	80.4+2.17

In Vivo Disease Control using Herbicides

Treatment of Rust Infected Millet with Paraquat and Glyphosate

Most of rust diseased millets treated with all doses (4.0 and 7.25 ml/l) of this herbicide did not show any recovery from the diseases. The plant parts especially those treated with these concentrations exhibited the injury symptoms similar to activity on contact. It was therefore not possible to differentiate between the pathogen activity symptoms and those resulting from the herbicide application injury. The 5.25 ml/l paraquat treatment showed a very slight effect with 2.33% survival, despite the injury exhibited by the seedlings (Table 3). The plant was able to shoot out fresh cotyledon despite the death of the leaves from the injuries by the herbicide application.

With the glyphosate treatments of rust disease, there was no recovery of the 10.00 ml/l treated millets, but the lower concentrations of 7.50 ml/l recorded very slight recovery, with 4.67%. The 5.25 ml/l treatment showed very slight effect on the disease control with 2.83% of the plants becoming alive after four weeks (Table 3). The survived plants were, however very stunted, showing serious height retardations.

Treatment of Damping off Infected Millet with Paraquat and Glyphosate

The 4.0, 5.25 and 7.25 ml/l treatment of damping off diseased millets was not effective with all concentrations of paraquat as presented in Table 3. The plant developed colour changes on the shoot before going ahead to die off. Survival of treated millets was not obtained. Damping off recorded 100% mortality without any survival.

Table 3: Percentage Survival of Millets due to Herbicide Disease Control

Treatment	Conc.(ml/l)	Damping off	Rust
Paraquat	Control	0.0+0.00	0.0+0.00
	4.0	0.0+0.00	0.0+0.00
	5.25	0.0+0.00	2..83+3.83*
	7.25	0.0+0.00	0.0+0.00
Glyphosate	Control	0.0+0.00	0.0+0.00
	5.25	0.0+0.00	2.33+1.53*
	7.50	0.0+0.00	4.67+0.92*
	10.0	0.00+0.00	0.00+0.00

Values are means of three replicates * = Significant change

IV. CONCLUSION

The antimicrobial activity of millet was recorded at highest concentration, but not at the lowest concentration. The tested pathogenic organisms were controlled at the test concentrations for damping off, but only slightly for rust, meaning higher herbicide concentrations may be needed. More preliminary studies on the feasibility of dual use of the herbicides will be very important.

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