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Original Research Paper

ASSESSMENT OF THE MICROBIAL POPULATIONS AND PHYSICOCHEMICAL COMPONENTS OF PARAQUAT TREATED SOIL

ABSTRACT

This study assessed the microbial population and physicochemical components of paraquat treated soils. The soil samples were treated with the low, recommended and high doses of paraquat respectively for a period of eight weeks at two weeks interval. Isolation and identification of the soil microbes as well as the determination of the physicochemical components (soil pH, organic carbon and organic matter) were carried out using standard procedures. The results of the study showed that, microbes such as *Staphylococcus* sp., *Micrococcus* sp., *Pseudomonas* sp., *Escherichia coli, Bacillus* sp., *Actinomycetes bovis, Actinomycetes Israeli, Streptomycetes* sp., *Aspergillus fumigatus, Aspergillus niger, Aspergillus flavus, Absidia corymbifera* and *Rhizopus stolonifer* were found present in the paraquat treated soils of different doses with the control soil samples having most of the fungal species isolated. The organic matter and carbon contents of the paraquat treated soils of different doses were at the 4th weeks after application (WAA) significantly ($P<0.05$) the highest whereas the pH of the soils was at 2nd WAA the highest compared to those of the other soils at different WAA. Different doses of paraquat at different WAA affect soil microbial populations as well the physicochemical components of the soil. So, the effects of paraquat on soil microbial population and physicochemical components depended on the concentrations used and the duration of application.

Key words: Paraquat, soil, microbial populations, physicochemical components

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1.0 INTRODUCTION

Herbicides are chemical compounds that are used to kill unwanted plants. These are used to clear waste grounds, industrial sites, railways and farm lands (Kelogg *et al.,* 2000). Prior to the wide spread use of chemical herbicides, cultural controls such as altering soil pH and fertility levels were used to control weeds. Mechanical control (including tillage) was also (and still is) used to control weeds (Kelogg *et al.,* 2000). Today, chemical herbicides is the indispensable instruments of reliable and intensive plant production and without their help, hunger and societal tensions would be more serious than the Ecotoxicological effect which they are intended (Bandala *et al.,* 2007). Numerous experiments have been conducted that demonstrated the potential positive impacts of the use of herbicides by farmers; for instance; maize yields double and production costs fell by 61% in Nigeria (Benson, 1982). Shave and Avav, (2004) also reported in Nigeria that the cost of weed control in rice with herbicides was 50% lower than hoe weeding.

In achieving the optimization of agricultural resources thus satisfying human needs and at the same time maintaining the quality of the environment and sustaining natural resources, the soil microbial community composition is of great importance, because they play a crucial role in carbon flow, nutrient cycling and litter decomposition, which in turn affect soil fertility and plant growth (Chauhan *et al*., 2006; Tripathi *et al.,* 2006; Pandey *et al*., 2007), and hence occupy a unique position in biological cycles in terrestrial habitat. The soil microbial population is considered as active nutrient pool to plants and plays an important role in nutrient cycling and decomposition in ecosystem (De-Lorenzo *et al*., 2001). A healthy population of soil microorganisms can stabilize the ecological system in soil (Chauhan *et al*., 2006) due to their ability to regenerate nutrients to support plant growth. Any change in their population and activity may affect nutrient cycling as well as availability of nutrients which indirectly affect productivity and other soil functions (Wang *et al*., 2008).

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However, despite the benefits of using these herbicides by farmers as noted by Bandala *et al.* (2007) and Shave and Avav (2004), the increased use of herbicides in agricultural soils causes the contamination of the soil with toxic chemicals, thus may exert certain effects on non-target organisms including soil microorganisms (Stanley *et al.*, 2013). In Nigeria, especially Mubi local government area of Adamawa State as well as most of the northern part of Nigeria where farming is the major occupation, strict observance of manufacturer's instructions on how to make use of these herbicides are not being adhered to as they are ignorant of its danger to the environment. Natural ways of controlling pests and pathogens of crops which are friendly to the environment have been forfeited by farmers for synthetic herbicides as the synthetic herbicides provide quick solutions to pests or pathogens problems as noted by Bandala *et al.* (2007). Therefore, there is need to occasionally assess the effects of herbicides on micro flora and other soil organisms as reported by Zaid *et al*. (2014) so as to know the measures to be taken. The use of paraquat as herbicide of choice in this study was as a result of it being the most widely used herbicide to control broad-leaf weeds and grasses especially in the study area.

2.0 MATERIALS AND METHODS

2.1 Study area

The study was carried out at Modibbo Adama University of Technology Yola, in the Department of Plant Science Botanical garden, Girei Local Government Area of Adamawa state, Nigeria.

2.2 Experimental design

Completely randomized block design (CRBD) was used to design the experimental field. For each treatment, there was one block which was replicated three times.

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2.3 Preparation of herbicides

The paraquat used was purchased from Jimeta Modern Market Yola, Adamawa State of Nigeria from the recommended dealer of herbicides. The preparation/concentration of the paraquat for application was prepared according to the manufacturer's recommendations. One-half and double of the recommended concentration for the herbicide was prepared also with a little modification for application at the rates of 0.3ml, 0.6ml, and 0.9ml (at 150 ml 10L) 0.6 L/ha (at 30 ml in 16 L sprayer). Each of the concentrations has three replications.

2.4 Soil Treatment

The application of the herbicide with the concentrations prepared above was done at two weeks interval over a period of eight weeks. The control blocks were not treated.

2.5 Sample Collection

The top soil samples were collected at the depths of up to 0-1 cm, 1-3 cm, and 5 cm respectively from each block of the field during each collection. It was then put into a sterilized polythene bags and were taken to the laboratory for isolation. The soil samples were then made free of large stones and plant debris using 2.0 mm mesh sieve and stored at 4^0C. The collection was done at two weeks interval for a period of eight weeks.

2.6 Isolation of Microorganisms

2.6.1 Isolation of Bacteria

Isolation of bacteria was performed by making serial dilution of the taken samples and the dilution that was used for studies were 10^{-2} and 10^{-4}. The dilution was spread in freshly prepared Nutrient agar medium plates. The media was weighed out and prepared according to the manufacture's specification, with respect to the given instructions and directions. The inoculated plates were then incubated at 37^0C for 24 – 48 hrs after cooling.

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After the incubation period, the pure cultures was obtained based on the color of colony and the pure cultures of the bacterial isolates was subjected to morphological and characterization tests to determine the identity of the bacteria isolates with reference to Bergey's Manual of Determinative Bacteriology as described by Cheesbrough (2006).

2.6.2 Isolation of Fungi

The isolation of fungi was performed by making pour plate method using Potato Dextrose Agar (PDA.) medium. The medium was weighed at 39g/L of distilled water and prepared according to the manufacture's specification, with respect to the given instructions and directions. The plates were then incubated at room temperature for 5 days. After incubation period, the pure cultures were obtained and the fungi were identified (firstly by staining a pinch of the pure culture placed on a slide with lacto-phenol stain and secondly, observing it under the microscope using 10X and 40X objective lens) through its micro and macro morphological structures (Guy, 2006).

2.6.3 Isolation of Actinomycetes

About 2.0ml, 5.0ml, 15ml of glycerol yeast agar and 1.0 g of potassium hydrogen phosphate (K_2HPO_4) were used for the isolation of actinomycetes and after incubation period of 7-14 days. The isolates were characterized based on colony.

2.7 Determination of soil pH

The pH of soil sample was determined using pH meter 3150 Jenway model according to the method described by Onyeike and Osuji (2003).

2.8 Determination of organic matter and organic carbon

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Organic carbon and organic matter in the soil was determined using Walkly and Black (1934) method.

3.0 RESULTS

3.1 Fungi, Bacteria and Actinomycetes species identified from paraquat treated and control (untreated) soil samples at two (2) weeks after application (WAA)

The results of the fungi, bacteria and actinomycetes identification in paraquat treated and untreated (control) soil samples at two weeks after application (WAA) showed that in paraquat low dose (PLD), paraquat recommended dose (PRD), paraquat high dose (PHD) and the control (CON), fungi, bacteria and actinomycetes such as *A. fumigatus*, *Pseudomonas* sp., and *A. israelis*; *A. flavus, E.coli* and *Streptomycetes* sp.; *A. corymbifera, Pseudomonas* sp. and *A. bovis; A. fumigatus, A. flavus, A. corymbifera, A. niger, R. stolonifer* and *Micrococcus* sp. and *Streptomycetes* sp. respectively were identified (Table 1).

3.2 Fungi, Bacteria and Actinomycetes identified from paraquat treated and untreated (control) soil samples at four (4) weeks after application (WAA)

At four (4) weeks after application, the fungi, bacteria and actinomycetes identification in paraquat treated and control soil samples showed that, in PLD, PRD, paraquat high PHD and the CON, fungi, bacteria and actinomycetes such as *A. flavus, Bacillus* sp. and *A. bovis*; *A. niger, E. coli* and *A. Israeli; R. stolonifer, Pseudomonas* sp. and *Streptomycetes* sp.; *R. stolonifer, A. flavus, A. niger, A. corymbifera, A. fumigatus* and *E. coli* and *A. bovis* respectively were identified (Table 1).

3.3 Fungi, Bacteria and Actinomycetes identified from paraquat treated and control soil samples at six (6) weeks after application (WAA)

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The result of the identification revealed the presence of fungi, bacteria and actinomycetes such as *A. niger, Staphylococcus* sp. and *A. bovis; A. flavus, Pseudomonas* sp. and *Streptomycetes* sp.*; R. stolonifer, E. coli* and *A. israeli; A. niger, A. flavus, R. stolonifer, A. coymbifera, A. fumigatus* and *Bacillus* sp. and *Streptomycetes* sp. in PLD, PRD, PHD and CON soil samples respectively (Table 1).

3.3 Fungi, Bacteria and Actinomycetes identified from paraquat treated and control soil samples at eight (8) weeks after application (WAA)

Identified from the PLD, PRD, PHD and CON soil samples were fungi such as *R. stolonifer, A. flavus, A. niger,* and *R. stolonifer; A. flavus, A. niger, A. corymbifera* and *A. fumigatus* respectively. *Bacillus sp, Pseudomonas* sp., *Bacillus* sp. and *E. coli* and *A. bovis, Streptomycetes* sp., *A. Israeli* and *A. bovis* respectively were the bacterial and actinomycetes species identified from the four soil samples (Table 1).

Table 1: Fungi, Bacteria and Actinomycetes species identified from paraquat treated and untreated soil samples

Week Interval	Treated Soil	Fungal Species	Bacterial Species	Actinomyetes Species
2nd WAA	Paraquat Low Dose	*Aspergillus fumigatus*	*Pseudomonas* sp.	*A. Israeli*
	Paraquat Recommended Dose	*Aspergillus flavus*	*E. coli*	*Streptomycetes* sp.
	Paraquat High Dose	*Absidia corymbifera*	*Pseudomonas* sp.	*A. bovis*
	Control (untreated soil sample)	*A. fumigatus* *A. flavus* *A. corymbifera* *Aspergillus niger* *Rhizopus stolonifer*	*Micrococcus* sp.	*Streptomycetes* sp.
4th WAA	Paraquat Low Dose	*A. flavus*	*Bacillus* sp.	*A. bovis*
	Paraquat Recommended	*A. niger*	*E. coli*	*A. Israeli*

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	Dose			
	Paraquat High Dose	*R. Stolonifer*	*Pseudomonas* sp.	*Streptomycetes* sp.
	Control (untreated soil sample)	*R. stolonifer* *A. flavus* *A. niger* *A. corymbifera* *A. fumigatus*	*E. coli*	*A. bovis*
6th WAA	Paraquat Low Dose	*A. niger*	*Staphylococcus* sp.	*A. bovis*
	Paraquat Recommended Dose	*A. flavus*	*Pseudomonas* sp.	*Streptomycetes* sp.
	Paraquat High Dose	*R. stolonifer*	*E. coli*	*A. Israelis*
	Control (untreated soil sample)	*A. niger* *A. flavus* *R. stolonifer* *A. corymbifera* *A. fumigatus*	*Bacillus* sp.	*Streptomycetes* sp.
8th WAA	Paraquat Low Dose	*R. stolonifer*	*Bacillus* sp.	*A. bovis*
	Paraquat Recommended Dose	*A. flavus*	*Pseudomonas* sp.	*Streptomycetes* sp.
	Paraquat High Dose	*A. niger*	*Bacillus* sp.	*A. Israelis*
	Control (untreated soil sample)	*R. stolonifer* *A. flavus* *A. niger* *A. corymbifera* *A. fumigatus*	*E. coli*	*A. bovis*

WAA: Week After Application

3.4 Effect of Paraquat Treatment on Fungal Count at 2 – 8 Weeks after Application (WAA)

At the 2nd WAA of PLD, PRD and PHD, the result showed that the fungal count was significantly higher ($P<0.05$) compared to those of the other WAA with 4.00, 3.00 and 4.00 x 10^4 cfu/mg respectively. However, that of PRD was not significantly different ($P<0.05$) from those of 4th – 8th WAA. The CON on the other hand, showed the highest fungal count at 6th

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and 8th WAA with 5.00 and 5.00 x 10^4 cfu/mg respectively which were significantly different ($P<0.05$) from those of 2nd and 4th WAA (Table 2).

3.5 Effect of Paraquat Treatment on Soil Bacterial Population at 2–8 WAA

The bacterial population was significantly the highest ($P<0.05$) at the 4th – 8th WAA of PLD, PRD and PHD with 33.20, 32.00 and 32.30 x 10^4 cfu/mg respectively. The control, however, was at 2nd and 6th WAA recorded the highest population of bacterial with 35.00 and 35.00 x 10^4 cfu/mg respectively (Table 2).

3.6 The Effect of Paraquat Treatment on Actinomycetes Population at 2 – 8 WAA

The population of actinomycetes at the 2nd WAA of PLD and PRD and even the CON were significantly the highest ($P<0.05$) with 2.50, 1.40 and 3.48 x 10^8 cfu/mg respectively. The same thing was observed for PRD at 6th WAA with 6.80 x 10^8 cfu/mg (Table 2).

3.7 The Effect of Paraquat Treatment on Soil Organic Carbon at 2 – 8 WAA

In Table 3, the application of paraquat doses recorded the most effective action on the soil organic carbon only at the 4th WAA with 1.34 %, 1.12 % and 1.12 % respectively except for its high dose at the 6th WAA which also had the highest effect (1.10) that was not significantly different ($P<0.05$) with that at the 4th WAA. The control, on the other hand, recorded the most effective action at the 6th and 8th WAA with 1.42 % and 1.43 % respectively (Table 3).

3.8 The Effect of Paraquat Treatments on Soil Organic Matter at 2 – 8 WAA

In Table 3, the application of the different doses of paraquat significantly ($P<0.05$) showed the most effective action at the 4th WAA with 1.93%, 1.93% and 1.84% respectively. The

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control, however, was only effective on the soil organic matter only at the 8th WAA with 2.46%.

3.9 The Effect of Paraquat Treatment on Soil pH at 2nd - 8th WAA

The application of paraquat doses was at the 2nd WAA recorded the most effective action with 7.39 %, 7.49 % and 7.49 % respectively. Similar action was also observed at 6th WAA for PHD with 7.49 % which was significantly the same ($P<0.05$) with that at the 2nd WAA. The control, also was at the 2nd WAA had the most effective action with 7.52 % (Table 3).

Table 2: Effect of paraquat on soil fungi, bacteria and actinomycetes populations

Week	Fungal count (10^4 cfu/mg)				Bacterial Population (10^4 cfu/mg)				Actinomycetes (10^8 cfu/mg)			
	PLD	PRD	PHD	CON	PLD	PRD	PHD	CON	PLD	PRD	PHD	CON
2nd WAA	4.00^{a}	3.00^{a}	4.00^{a}	2.00^{b}	24.00^{b}	16.00^{c}	9.80^{c}	35.00^{a}	2.50^{a}	2.70^{d}	1.40^{a}	3.48^{a}
4th WAA	3.00^{ac}	2.00^{a}	1.00^{b}	3.00^{b}	33.20^{a}	15.20^{c}	12.30^{b}	16.40^{b}	0.55^{c}	4.00^{c}	0.12^{c}	0.15^{c}
6th WAA	3.00^{ac}	2.00^{a}	2.00^{b}	5.00^{a}	21.40^{bc}	32.00^{a}	32.30^{a}	35.00^{a}	1.32^{b}	6.80^{a}	0.17^{c}	2.39^{b}
8th WAA	2.00^{bc}	3.00^{a}	2.00^{b}	5.00^{a}	19.00^{c}	20.30^{b}	9.00^{c}	9.50^{c}	0.82^{c}	5.30^{b}	1.23^{b}	2.76^{b}

Key: Means with the same superscript(s) along the column are not significantly different at $P<0.05$.

PLD: Paraquat low dose; **PRD:** Paraquat recommended dose; **PHD:** Paraquat high dose; **CON:** Control; **WAA**= Week After Application

Table 3: Effect of paraquat treatment on soil organic carbon, Organic matter and soil pH

Week	Organic carbon (%)				Organic matter (%)				Soil pH			
	PLD	PRD	PHD	CON	PLD	PRD	PHD	CON	PLD	PRD	PHD	CON
2nd WAA	0.38^{d}	0.55^{d}	0.38^{c}	0.95^{c}	0.65^{c}	0.95^{d}	0.62^{d}	1.63^{d}	7.39^{a}	7.49^{a}	7.49^{a}	7.52^{a}
4th WAA	1.34^{a}	1.12^{a}	1.12^{a}	1.07^{b}	1.93^{a}	1.93^{a}	1.84^{a}	2.33^{c}	6.49^{b}	6.81^{ac}	6.84^{ac}	6.83^{ac}
6th WAA	1.01^{b}	0.99^{c}	1.10^{a}	1.42^{a}	1.62^{b}	1.89^{b}	1.57^{c}	2.44^{b}	5.73^{cd}	6.35^{bc}	7.49^{a}	6.55^{bc}
8th WAA	0.95^{c}	1.06^{b}	0.94^{b}	1.43^{a}	1.63^{b}	1.82^{c}	1.62^{b}	2.46^{a}	6.23^{bd}	6.94^{ac}	6.36^{bc}	6.74^{bc}

Key: Means with the same superscript(s) along the column are not significantly different at $P<0.05$.

PLD: Paraquat low dose; **PRD:** Paraquat recommended dose; **PHD:** Paraquat high dose; **CON:** Control; **WAA**= Week After Application

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4.0 DISCUSSION

In this study, assessment of fungal species in paraquat untreated (control) soil samples from the 2 – 8 WAA presents fungal species such as *A. fumigatus*, *A. flavus*, *A. niger*, *A. corymbifera* and *R. Stolonifer* which were found common in all the control soil samples. Similarly observation was made by Dagze *et al.* (2016) in their control soil samples when they determined the effect of atrazine on soil microbes. This occurrence could be attributed to the fungi being well adapted to the soil conditions or probably the health status of the soil as similarly reported by Zain *et al*. (2013) who associated soil microbial activity and content to soil health. Also, the similarity in fungal species isolated in the control soil samples of paraquat (of this study) and atrazine (of Dagze *et al.*, 2016) treated soils could be as a result of these studies being carried out in the same study area with probably the same environmental conditions as such factor according to Bolter *et al.* (2006) affect soil microbes. On the other hand, fungal species such as *A. fumigatus*, *A. flavus*, *A. corymbifera*, *A. niger* and *R. stolonifer* were found present in the paraquat treated soils with *A. niger*, *A. flavus* and *R. stolonifer* being the commonest especially from the 4-8th WAA. This finding was supported by the findings of Ayansina and Oso (2006) who reported the presence of *A. niger*, *A. flavus* and *Penicillium* sp. in their herbicides treated soil. However, their findings differ in one of the fungal species identified probably as a result of the differences in the type of agrochemicals, concentrations, mode of applications, environmental conditions and herbicides combination used as these according to Subhani *et al.* (2000) and Zain *et al.* (2013) affect soil's microbes content. Apparent from the assessment of the type of fungal species present in the untreated (control) soils of this study was the fact that fungal species, *A. corymbifera*, was not present in the paraquat treated soils especially from 4th – 8th WAA. This could be that the paraquat had a fungicidal effect on the fungi species.

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The results of the identification of bacteria and actinomycetes present in the treated and control (untreated) soil samples showed that, bacteria such as *Staphylococcus* sp., *Bacillus* sp., *Streptococcus* sp., *Pseudomonas* sp., *E. coli* and *Micrococcus* sp. were present in both the treated and untreated (control) soil samples with *Pseudomonas* sp. and *E. coli* being the most common in occurrence in the soil samples from the 2nd – 8th WAA. On the other hand, actinomycetes such as *A. bovis*, *A. israelis*, *Streptomycetes* sp. were identified and even the commonest in all the WAA.

These bacteria and actinomycetes species identified, differ across the number of WAA of the treatments as well as the doses probably as a result of the effect of the chemicals on them. This was so because herbicides could adversely affect soil microbes depending upon the application rate/dose and the type of herbicide used (Ayansina and Oso, 2006; Sebiomo *et al.*, 2011). The bacterial species identified in the treated and control soil samples of this study were similarly reported by Stanley *et al.* (2013) in the control and paraquat treated soils. However, some of the bacterial species identified in this study such as *Staphylococcus* sp., *Streptococcus* sp., and *E. coli* were not reported in their findings probably as a result of the differences in concentration used, period of application and collection of samples for assessment and the depth at which the soil samples were collected.

The effect of paraquat treatments on soil fungal count at the 2nd WAA of PLD, PRD and PHD showed a higher fungal count compared to those of other WAA. Similar report was given by Stanley *et al.* (2013) who reported a decrease in the fungal count of the soil treated with butachlor, pyrazosulfuron and paraquat with passage of time from 7th to 28th day after treatment. The decrease of the fungal count observed in this study could be due to the adverse effect of the herbicide on the fungal component of the soil. This was proven by the fungal count of the control as the fungal count was higher especially at 6th and 8th WAA.

Bacterial population was low for most of the paraquat doses at the 2nd WAA, but increased at other WAA. Ayansina and Oso (2006) discovered that higher concentrations of herbicide treatments resulted in much lower microbial counts when compared to soils treated with recommended doses. This study somehow agrees with the above statement because the bacterial population of the control soil samples had the highest compared with those of paraquat treated soils.

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Actinomycetes population was high at 2nd WAA of PLD, PRD and the control as well as at the 6th WAA of PHD. This could be influenced by the factors such as temperature, pH, organic carbon content, aeration and moisture content of the soils as reported by Arifuzzaman *et al.* (2010). Similar result was published by Stanley *et al.* (2013) who reported a significant increase in actinomycetes population from 7th to 28th day as a result of herbicide application.

The behavior of most herbicides is generally influenced by the content of organic carbon (Torrents and Jayasunda, 1997). The phenomenon of herbicide leaching was primarily related to the adsorption of herbicides in the different soils, mainly due to their contents of fulvic-acids, humus in the organic fraction of the soils, followed by parameters such as the cat-ion exchange capacity, acid dissociation constant (Alister *et al*., 2011). Variation in the effect of paraquat treated soils on organic carbon content was observed with respect to the doses of the herbicides and the weeks after application. Same was observed for the effect of paraquat on the soil organic matter content as well as pH of the soils. This could be due to vigorous microbial activities in the soil (Greaves *et al.*, 1976).

5.0 CONCLUSION

Different doses of paraquat at different WAA affect soil microbial populations as well the physicochemical components of the soil. So, the effects of paraquat on soil microbial populations and physicochemical components depended on the concentrations used and the duration of application. It also determines the type of microbes present in the soil by getting rid of those that cannot withstand its effects, in this way allowing only the resistible species.

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