Bajopas Volume 10 Number 1 June, 2017

http://dx.doi.org/10.4314/bajopas.v10i1.32

Bayero Journal of Pure and Applied Sciences, 10(1): 223 – 226
Received: June, 2015
Accepted: November, 2016
ISSN 2006 – 6996

THE EFFECTS OF TEMPERATURE AND RELATIVE HUMIDITY ON THE GROWTH OF THREE ISOLATED FUNGI FROM RICE (*Oryza sativa* L.) SEEDLINGS IN DADIN KOWA IRRIGATION SCHEME, DADINKOWA, GOMBE

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ABSTRACT

The effects of temperature and relative humidity on the growth of three isolated fungi (Aspergillus parasiticus, Altenaria alternata and Thielavia terricola) associated with rice seedlings rot in Dadin Kowa Irrigation Scheme, Gombe, Nigeria were investigated. Temperature in the ranges of 10°C, 15°C, 25°C, 30°C, 35°C and 40°Cwere used to determine the temperature effect on the growth of these fungi. These fungi were also cultured on 100, 91, 80, 59.5, 47 and 32.5 % relative humidity. Highest growth of these fungi was obtained at 25°C and 30°C temperatures. The fungi showed highest growth at 80 and 91% relative humidity. The growth of these fungi was observed to increase with increase in relative humidity and vice versa. Statistical application for Sciences (SAS) was used to analyze the data generated and the least significant difference, was used to separate the means. There were significant differences (P≤0.05) in the growth of these fungi at different temperature and relative humidity regimes.

Keywords: Temperature, Relative Humidity, Growth, Rice, Fungi, Dadin Kowa

INTRODUCTION

Rice (*Oryza sativa* L.) is a monocotyledonous cereal crop which belongs to the grass family Poacea (formerly graminae). In Nigeria rice (grown on 1.77 million ha) ranks sixth after sorghum (4.0 million ha), millet (3.5 million ha), cassava (2.0 million ha) and yam (2.0 million ha), but if placed on a social scale, it can as well be ranked first because it is no longer just a mere festival meal, but the staple of most homes in urban and rural areas (Ekeleme *et al.,* 2008)

Several studies were conducted on the effect of environmental factors on the growth of fungal pathogens (Malik and Singh 2004; Kim and Xiao, 2005). Environmental factors such as relative humidity and temperature were reported to have a significant influence on the growth and development hence virulence, of a variety of fungi. Growth rate of fungi varies depending on temperature and relative humidity. The optimum growth temperatures for the majority of fungi studied was found to fall between 25˚C and 30˚C and above 40˚C the growth was poor and in some cases mortality may occur (Sharma and Razak, 2003).Bristone *et al.* (2011) reported that temperature ranges of 10 – 25° C inhibited the growth of *Penicillium expansum* and *Rhizopus stolonifer*. Channya (1991) also found that the presence of moisture (Relative Humidity) predisposed plant tissues to microbial infection, moisture, were required for spore germination on the host surface and also protects the germ tubes from desiccation before invading the host tissues. He also detected that temperature range of 35 - 40° C affected the growth of many fungi as regards their ability to cause rotting. Chimbekujwo (1994) also reported that potato rot was fast between temperature range of 25 - 30° C and relative humidity range of 32.5 – 100 %. Reis *et al.* (2006) on the study of effect of lesion age, humidity and fungicide application on sporulation of *Alternaria alternata,* the cause of brown spot of Tangerine, reported that the number of conidia produced was low at 74 % relative humidity and greater at 80 % relative humidity. The percentage relative humidity of lesion producing conidia was very low at 74 %, increased at higher relative humidity of 80 - 85 % and declined slightly at 100 %.

However, little information is available on the influence of environmental factors on the growth of the fungi, *Aspergillus parasiticus, Altenaria alternata* and *Thielavia terricola*. Therefore, the main objective of the study was to investigate the effect of temperature and relative humidity on the growth of these fungi. This may contribute in reducing heavy losses incurred annually by rice farmers at the irrigation schemes as a result of the devastating effect of these fungi on rice seedlings both at harvest and post-harvest levels.

MATERIALS AND METHODS

Samples Collection and Processing

Diseased rice seedlings were collected from rice farms in the Dadin kowa irrigation scheme and transported to the Biological Science Department, Gombe State University. The diseased samples were surface sterilized and cut into pieces of root portion, stem portion and head portion separately using sterilized hand knife. The pieces were transferred onto Petri dishes containing potato dextrose agar media using sterile spatula which was sterilized using Bunsen flame and dipped it into methylated spirit.

Isolation and Identification of the Fungi

A portion of the diseased rice plant was cut into smaller pieces with sterile hand knife on a sterile wooden surface and the pieces were transferred into sterile Petri-dishes with a sterile spatula which was flamed over a Bunsen flame and dipped inside methylated spirit (Thomas, 1979). The cut piece were surface-sterilized with 0.01 % mercuric chloride for 30 seconds, rinsed in five changes of sterile distilled water, and was blotted dry with sterile Whatman No. 1 filter papers. Cut pieces were plated aseptically on 9 cm Petri dishes containing solidified PDA to which 0.3 g tetracycline was added to inhibit the growth of bacteria. Solidified plates were incubated at room temperatures (28 - 32^o C) for 4 days. Fungal colonies growing from the incubated plates were sub-cultured into fresh PDA media until pure cultures were obtained. Microscopic examinations were carried out after examining the colony characteristics. A sterile needle was used in taking a little portion of the hyphae, containing spores onto sterile glass slide stained in lacto phenol cotton blue and examined under the microscope for the fungal structures (Frazier, 1978). The morphological, and cultural characteristics observed under the microscope was compared with structures in Alexopoulus and Mims (1986), as well as Snowdon (1990).

Effect of Relative Humidity on the Growth of the Fungi

Six different relative humidity regimes were obtained as proposed by Winston and Bates (1960). Such relative humidity was obtained by dissolving each salt at 30^{o}C until the saturation state was reached. The six different relative humidity regimes 32.5 %, 47 %, 59.5 %, 80 %, 91 %, and 100 % were obtained using desiccators by dissolving the following salts: Magnesium chloride ($MgCl_{2)}$, calcium nitrate ($Ca(NO_3)_2$), ammonium nitrate (NH_4NO_3), ammonium sulphate (NH_4SO_4) and potassium nitrite (KNO_2) above their saturated solutions, and distilled water respectively. Kim and Xiao (2005) method was adopted whereby (2 mm) of 3 days pure culture of the isolated fungi were inoculated on a sterile Petri dish containing PDA medium and arranged in the upper chamber of the desiccators containing the desired salts and allowed to stand for 4 hours to attain the relative humidity required. A plate containing the isolated organisms was incubated in desiccators containing each relative humidity regime. This was allowed for 24hours after which the progress of radial growth was measured for four consecutive days. The experiment was replicated four times.

Effects of Temperature on the Growth of Fungi

Six temperature regimes used for this study were 10, 15,25,30,35 and 40^{o}C. The variations of temperature were obtained by setting the incubators to the required temperatures. A 2mm diameter of the youngest of 3-day old colony of the fungus was inoculated into a solidified (PDA media) and transferred into the incubator which was initially set at the required temperature regimes for four days. The experiment was replicated four times for each of the temperature regimes.

RESULTS AND DISCUSSION

The results of the growth of the isolated fungi (*Aspergillus parasiticus, Altenaria alternata* and *Thielavia terricola)* are presented in Table 1.The temperature regimes tested supported the growth of the fungi. However,the results of the growth of the three species of fungi isolated at varying regimes of 10^{o}C, 15^{o}C, 25^{o}C, 30^{o}C, 35^{o}C, 40^{o}C showed an increase in radial growth mean value from 10^o C being the lowest growth temperature to 35^o C as the optimum temperature for the growth of the organisms. *Aspergillus parasiticus* and *Alternaria alternate* temperature has a highly significant effect on their growth and development as shown by their P –values (0.0012 and 0.002) compared to $P \le 0.05$.Thus,the lower the P-value for a given isolated organism over the temperature regime it was subjected the higher the significant the temperature regime will be for their growth and development. However, their respective radial growth mean values stood at 2.03 mm and 2.07 mm respectively. Statistical analysis showed a highly significant effect of temperature on the isolated fungi ($P \le 0.05$) at 10^{o}C, 15^{o}C and 40^{o}C.Temperature also had a significant effect on the growth and development of *Thielavia terricola* as shown by its P-value (0.0224) and its radial growth mean value stood at 2.04 mm.

The results of the three species of the isolated fungi at varying relative humidity regimes of 32.5 %, 47 %, 59.5 %, 80 %, 91 % and 100 %; showed that the effect of relative humidity on *Alternaria altenata* was highly significant as its P- value (0.02) a value below the set P- value (0.05).Therefore the lower the P-value the higher the significant is the effect of the relative humidity on the isolated fungi, also the radial growth mean value stands at 2.03 mm. On the other hand *Aspergillus parasiticus* was significantly affected by the relative humidity as shown by its P- value (0.06) and its radial growth mean value was 2.38 mm. At the relative humidity of 32.5 %to 100 % the radial growth mean value of the *Aspergillus parasiticus* performed well. While *Thielavia terricola* responded to some relative humidity regimes of 47 %, 59.5 % and 91 % and failed at 32.5 %, 80 % and 100 %.

Table 1: Effect of Different Temperature Regimes on the Radial Growth in Millimeter (mm) of the Isolated Fungi

Temp. (^{0}C)	*Altenaria altanata*	*Aspergillus parasiticus*	*Thielavia terricola*
10	2.00	2.10	2.00
15	2.00	2.10	2.10
25	2.05	2.00	2.05
30	2.00	2.08	2.10
35	2.03	2.13	2.00
40	2.10	2.00	2.00
Mean	2.03	2.07	2.04
LSD	0.05	0.07	0.08
P-Value	0.0012 **	0.002 **	0.0224 *

Values represent means of quadruplicate Petri dish
Key:
** = Highly significant at ($P \leq 0.05$), * = Significant at ($P \leq 0.05$)

Table 2: Effect of Different Relative Humidity on the Radial Growth in Millimeter (mm) of Isolated Fungi

R.H (%)	*Alternaria alternata*	*Aspergillus parasiticus*	*Thielavia terricola*
32.5	2.15	2.20	2.00
47	2.63	2.45	2.18
59.5	2.25	2.43	2.05
80	2.13	2.60	2.00
91	2.25	2.34	2.10
100	2.20	2.25	2.00
Mean	2.27	2.38	2.05
LSD	0.29	0.27	0.15
P-Value	0.02**	0.06*	0.11^{ns}

Values represent means of quadruplicate Petri dish
Key: ** = Highly significant at ($P \leq 0.05$); * = Significant at ($P \leq 0.05$)
ns = Not significant at ($P \leq 0.05$)

In this research, the three (3) isolated fungi responded differently to the temperature ranges, their growth rate on the rice seedlings showed that the optimum growth temperature for *Alternaria alternata, Aspergillus parasiticus* and *Thielavia terricola* were between the ranges of 10^{o}C to 35^{o}C. This supports the report by Chimbekujwo (1994) in his studies of pawpaw rot. However,a lower temperature of 10^{o}C to 15^{o}C tends to lower the growth of some of the isolated fungi (*Alternaria alternata, Aspergillus parasiticus* and *Thielavia terricola*). This tallies with the findings of Bristone*et al.* (2011).In corroboration with this study, Gulzar *et al.* (2011) found that the best temperature range for maximum growth of three fungi (*Pyricularia grisea, Helminthosporium oryzae* and *Rhizoctonia solani*) of paddy seedlings in the valley of Kashmir in India fell between 5^{o}C and 35^{o}C.This shows generally that the fungal flora virulence to infect rice seedlings irrespective of their taxonomic class is temperature dependent and the range is between 5^{o}C to 40^{o}C.

This present study has shown that rice seedling diseases caused by the three (3) species of isolated fungi are favored within the relative humidity range of 32.5 % to 59.5 % and 100 % for some of the isolates (*Alternaria alternata, Aspergillus parasiticus,*) while *Thielavia terricola* showed no radial growth at 80 % relative humidity and 100 % relative humidity. This indicated that humid atmosphere favors the growth and development of the organisms hence, the prevalence of the diseases as supported by Channya (1991) who showed that presence of moisture (relative humidity) predisposed plant tissues to microbial infections. However, the failure of *Thielavia terricola* to grow under higher relative humidity may not be unconnected with the fact that relative humidity value of 80 % and above entails rainfall meaning that only fungal flora that has inherent hydrophilic character can thrive under such high moisture condition. Reis *et al.* (2006) reported that the number of conidia produced was low at 74 % relative humidity and greater at 80 % relative humidity, but declined slightly at 100 % relative humidity, this agrees with the findings of this study whereby after progressive increased in radial growth of the isolated fungi over relative humidity regimes of 32.5 – 80 %, it declined at 91 % and 100 % relative humidity. This is to indicate that higher relative humidity entails rainfall under which only aquatic fungi and hydrophilic fungal flora can survive.

CONCLUSION

This study has shown that *Alternaria alternata, Aspergillus parasiticus,* and*Thielavia terricola* were responsible for the rice seedling diseases at Dadin Kowa Irrigation Scheme and that environmental factors such as temperature and relative humidity have effects on the development of the diseases;

It is apparent from the study that low temperature and low relative humidity do not support the growth of *Alternaria alternata, Aspergillusparasiticus,* and*Thielaviaterricola* Therefore, it is highly recommended that rice seedlings prior to transplanting should be stored at low temperature and low relative humidity to avoid infection due to this isolated fungi and this may contribute significantly in controlling the heavy losses our indigenous farmers incurred because of this fungi both at harvest and post-harvest level

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