Application of Nestedness Pattern to Germination Studies on Maize seeds

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ABSTRACT

The effect of systemic fungicide on germination of seeds is an important area to recommend the particular fungicide for farmers. In this regard the tricyclazole fungicide a systemic fungicide which is used in the control of blast diseases of paddy is tried to the maize seeds (MHM9001) a cultivar which is used in Karnataka and the tricycazole is sold as a common systemic fungicide for the various seeds. The seeds were treated and allowed to germinate and after 15 days the seeds were subjected to various morphological and biochemical parameters and the data obtained was subjected to 't' test but the values were varied with concentrations so the result were subjected to nestedness pattern which describes about species abundance and assemblages. Nestedness is a measure of order in ecological system referring to the order in which the number of species is related to the area or other factors.

A presence absence matrix was formed from the't' test table. Values of T=0° is a perfectly nested assemblage while higher values represent disordered assemblages. The temperature calculated for the test was 29.81° indicating a slightly warmer matrix or disordered assemblage.

Perfectly nested parameters were vigor index, percent germination, Leaf weight, conductivity, total proteins, DNA content, purity, DNA and RNA. These values were lower than the control at all the concentrations used.

***Keywords*:** Maize seeds, t test Nestedness.

INTRODUCTION

The treatment of various seeds with fungicides for the prevention of plant diseases is becoming more common as our knowledge of the life history of the numerous injurious parasitic fungi becomes more extended. It is clear that in those cases where chemicals are used to destroy fungous spores attached to the seed, the latter should pass through the treatment with as little loss of germinating power as possible. It is the object of the experiments detailed in the present study to determine the effect upon the germinating power of seeds produced by treatment with such chemicals as are likely to be used as fungicides. The treatment usually employed, adopted here as reported in the following pages, is that of

soaking the seed for a definite period in a solution of some chemical of known strength. It is obvious that seeds with thick seed coats would resist the action of a solution by virtue of the protective coating. Since it was the purpose to show the effect of the chemicals upon the vital processes in the seed rather than the resistance of the seed coat, such seeds were chosen as would readily absorb the chemicals

Triazoles are a group of fungicides and plant-growth-retarding chemicals, which contains three conserved nitrogen atoms within a five-member ring. Several of these compounds, including paclobutrazol, uniconazole, tetraconazole, and triadimefon, cause remarkable growth responses in plants. Changes caused by triazoles are mediated through cytochrome P-450 group of enzyme inhibition (Sopher, et al., 1999; Taiz and Zeiger, 1998; Zhu et al., 2004). There is evidence that triazoles applied to plants could be a means to alleviate water stress. In fact, plant responses such as increased ABA, proline, antioxidants and stomatal closure were observed after triazole treatment which appears to mimic some drought avoidance or tolerance mechanisms. This has been reported in wheat (Berova and Zlatev, 2003); (Gilley and Fletcher, 1997), Tomato (*Lycopersicon esculentum*) (Still and Pill, 2004), perennial rye grass (*Lolium perenne*) (Jiang and Fry, 1998), and silver maple (*Acer saccharinum*) (Marshall et al., 2000).

MATERIALS AND METHODS

The hybrid maize seeds (MHM 9001) was purchased from Mudra seeds Pvt limited, Hyderabad, India sold in local agricultural agency in Mysore district, Karnataka, India. The seeds were already treated with thiram for long time storage. These seeds were selected randomly from the seed lot and treated with Tricyclazole at 0.1%, 0.2% and 0.3%. The seeds were soaked in the fungicidal solution of above concentrations for 24 hours. Later the seeds were allowed to germinate on germination on top of the paper method for 15days. (Nene and Thapliyal,1993). The seeds were then analyzed for Morphological and Biochemical parameters. Three sets for each treatment ware maintained.

Seedling vigor index (SVI) was calculated following modified formula of Abdul-Baki and Anderson (1973); Conductivity test is used to quantify the leakage of electrolytes from the seed coat. (Hendricks, S.B and Taylorson, R.B. 1976); Chlorophyll was extracted in 80% acetone and determined by Arnon method. (Arnon,1949); Reducing sugars were estimated by Dinitrosalicylic acid method (Miller,1972) ; Total carbohydrates and Starch estimation was done by the Anthrone method (Hedge,1962) ; Total Proteins were estimated using Lowry's method (Lowry *et al* 1951) ; Isolation of DNA was done by chloroform and isoamylalcohol mixture in 24:1 ratio (Murmur,1961) purity of DNA was determined (Hillary Luebbehusen 2004); DNA was estimated by DPA method (Burton, 1956) and RNA estimation was done by Orcinol method (Bial,1902). The data obtained were subjected to Statistical analysis of standard 't'test to arrive at precise conclusion. The procedures for biochemical estimations was collected from "Biochemical Methods" by Sadasivam, S and Manickam, A (2008)

RESULTS

Maize seeds (MHM 9001) were treated with tricyclazole with varied concentrations (0.1%, 0.2% and 0.3%) for 24 hours. The treated seeds were transferred to germination

papers and between papers method were used and allow germinating for 15 days. The seeds were then analyzed with various biochemical and morphological parameters and the values obtained were subjected to "t" test and then the results were subjected to nestedness pattern which explains about the orderness of the data and assemblages of species. A presence absence matrix was prepared with the results of the "t" test. Here the values lesser than the control was treated as positive and denoted as presence (1) and the values which were higher than the control were treated as negative and denoted as absence (0) is employed so as to see the effect of fungicide on the germination of maize seeds. Here the values lesser than control was more than the values higher than the control. The data obtained were subjected to nestedness software and the results obtained were analyzed.

The concentrations were arranged in vertically and various parameters are arranged horizontally. The rows and columns emerge from the data were arranged so as to minimize unexpected positive and negative effects. In usual cases few matrices will prove to be perfectly nested, all matrices can be packed to a state of maximal nestedness. The top most parameters in the generated figure are judged to be most common effects. Similarly, the parameters in left are those to be perfectly nested and which can be easily applicable to any sets of experiments.

The most important aspect of the software is the matrix temperature calculation. Statistical stochasticity is a concept closely related to heat, information, order, or disorder. The calculated matrix in the data set measures, the reaction of maize cultivar against the treatment. The matrix uses the distribution of unexpected results in the presence or absence in the matrix. To calculate matrix temperature, unexpectedness must be defined. In a perfectly nested matrix, the set of results of any experiment will be a perfect subset of all the results. The hypothetical line that separates the occupied area of matrix from the unoccupied area is termed as extinction (in this case unexpected results). Further for every unexpected negative response there will be an unexpected positive response below it. The matrix temperature is a measure of the extent of the effect of the treatment.

1. State occupancy calculation:

The nested distribution imply that the cell most likely to be occupied in any matrix will be the top left one, that is, the most ubiquitous results will virtually always appear in the most resistant cultivar. Similarly, the cell least likely to be occupied will be the bottom right cell where the most absent matrices are likely to be present. absent matrices are least likely to be occupied will be the bottom right. As matrix temperature increases, unexpected positive and negative results begin to appear in those cells nearest to the boundary line, where unexpectedness values are lowest.

2. Probability calculation:

The probability that any distribution pattern might be randomly produced can be estimated using Monte- Carlo techniques using a run value of 100. the extent to which the characteristic temperature of the matrix deviated from 100 depends on the size, shape and degree of fill of the matrix. Highly rectangular matrices produce colder characteristic matrices.

3. Idiosyncratic temperatures:

Idiosyncrasies often indicate unmarked heterogeneity in datasets. This is because the treatment brings about different response of cultivars. Nestedness analysis was carried out using the presence absence matrix (concentration of fungicide in rows and parameters in columns). The reorganized nestedness pattern matrix pack for the data set is presented in table2. The matrix fill, idiosyncratic temperature by island and idiosyncratic temperature by species are represented in fig1.(islands represents the 3 concentrations of the fungicide and species represents the 20 parameters analyzed.). The analysis of the data shows highly significant nested pattern (T<29.81°= 6.55e-01(0.41e) matrix fill 48.1%) which indicates a slightly warm matrix. However matrix fill less than 50%, a perfectly ordered matrix forms a concave line in the upper left corner of the figure. This is an indication of the nested pattern or the conduct of experiments and treatment of fungicide do have a significant effect, but the results are not perfect for a Monte Carlo run count of 100 randomized matrices. The numbers of nested reactions were high in comparison to unexpected reactions (idiosyncratic reactions). The matrix for island (fungicide concentration) is presented in table 3 and the matrix for species (morphological and biochemical parameters) reorganization is given in table 4.

The probability of state occupancy indicates that expected values (97 to 100%) top left were high, but unexpected results were even higher (bottom right). The figure indicates that negative effects of Tricyclazole treatment on maize (MHM 9001) were more than positive.

The fact that the matrix is nested (48.1%) for different morphological, biochemical in seeds is an indication that the responses observed are quite significant. The top left parameters in the significantly cold temperature are the resistant cultivars of Zea mays. Idiosyncratic results are less. The nestedness pattern usually used for species distribution can also be used in the analysis of experimental data.

DISCUSSION

The effect of Tricyclazole on maize seeds was the main area of this study. The seeds were treated with fungicide of varied concentrations like 0.1%, 0.2% and 0.3%. The treated seeds were analyzed with morphological and biochemical parameters. The values were subjected to “t” test. A presence–absence matrix (matrix having sites in rows and species in columns, with 1 for presence and 0 for absence) was constructed for nestedness calculation. The degree of nestedness was calculated using the nestedness calculator. The metric *T* of the ‘nestedness calculator’ measures the extent of unexpected presence and absence in a maximally packed matrix, where sites are arranged in rows in descending order according to their species richness, and species are arranged in columns according to the number of sites on which they occur. The metric $T = 0°$ for a set of perfectly nested assemblages, and $T = 100°$ for completely disordered ones. The null hypothesis that *T* is not lower (i.e. more nested) than expected by chance, was tested using Monte Carlo permutations. The parameters that were nested perfectly were vigor index, percent germination, total proteins, leaf weight, conductivity, DNA content, DNA and RNA content of the seeds treated with all the concentrations of the fungicide and this included both morphological and biochemical parameters. The vigor index, percent germination, leaf weight, conductivity, total proteins,

DNA, DNA content, RNA and DNA were the parameters which were greatly affected with the treatment of the fungicide. All these parameters were greatly affected hence it showed positive result, i.e., it had positive effect on germination as well as metabolism of Zea mays. The other parameters stood next in the order of their effect on germination and metabolism of maize seeds. The above mentioned parameters had effect in all the concentrations, while 0.2% is mostly important in these effects. Hence the tricyclazole though it is a systemic fungicide it cannot be used in the germination of maize seeds. This fungicide is not exhibiting any stress conditions to the germination of sorghum seeds. The concentrations also played a major role in determining the changes. The mid concentration (0.2%) stood at top while the lowest concentration (0.1%) at last. The 0.3% concentration had medium effect in the germination and other parameters.

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Table 1. Effect of Tricyclazole on the metabolism of Zea mays Morphological parameters

Sl No	Parameters	Control	0.1%	0.2%	0.3%
01	Vigor Index	2701	474.2***	1084.1 ***	788.7***
02	Shoot Length	5.2	9.27*	10.02***	7.48**
03	Root length	18.2	13.5***	8.18***	18.82*
04	% Germination	90.00	20.00**	60.00	30.00**
05	Fresh Weight	6.8	8.5*	10.4***	6.2***
06	Seed Weight	3.33	5.53*	5.200 **	4.200
07	Leaf weight	1.17	0.73	0.46*	0.493*
08	Root Weight	2.25	2.30	4.8**	1.3*
Biochemical Parameters					
09	Conductivity	352.00	246.33***	285.67**	82.33
10	Chlorophyll a	0.015	0.023*	0.008*	0.030*
11	Chlorophyll b	0.008	0.022*	0.007	0.030*
12	Total chlorophyll	0.025	0.045***	0.015*	0.046
13	Reducing sugars	200	233.3	300	200
14	Total Carbohydrates	300	283.3	500	723.3 *
15	Nonreducing sugars	900	906.6	643.33 *	1070 *
16	Total Proteins	0.43	0.10	0.24	0.13 *
17	DNA quantification	0.13	0.023*	0.10	0.03
18	DNA content	0.64	0.5*	0.53	0.53
19	DNA	120	60*	30*	50*
20	RNA	190	156.7	130	120*

Note: *** Values which are extremely significant.
** Values which are very significant
* Values are significant

Table 2. Matrix reorganization vector for the treatment of fungicide on Maize seeds.

Sl No	Parameters	0.1%	0.2%	0.3 %
1	Vigor Index	1	1	1
2	% Germination	1	1	1
3	Leaf weight	1	1	1
4	Conductivity	1	1	1
5	Total Proteins	1	1	1
6	DNA content	1	1	1
7	DNA purity	1	1	1
8	DNA	1	1	1
9	RNA	1	1	1
10	Root length	1	1	0
11	Total Chlorophyll	0	1	0
12	Chlorophyll b	0	1	0
13	Nonreducing sugar	0	0	1
14	Total Carbohydrates	0	1	0
15	Root weight	0	0	1
16	Fresh weight	0	0	1
17	Chlorophyll a	0	1	0
18	Shoot Weight	0	0	0
19	Reducing sugar	0	0	0
20	Shoot length	0	0	0

Table 3. Island Reorganization Vector (Fungicide concentration)

Current Row Position	Original Row Position
1	2(0.2%)
2	3(0.3%)
3	1(0.1%)

Table 4. Species Reorganization Vector (parameters)

Current Row Position	Original Row Position
1	1(Vigor Index)
1	4(% germination)
1	7 (Leaf Weight)
1	9 (Conductivity)
1	16 (total Proteins)
1	17 (DNA)
1	18(DNA content)
1	19(DNA Estimation)
1	20(RNA Estimation)
1	3(Root Length)
1	12(Total Chlorophyll)
1	11(Chlorophyll b)

Table 4. Species Reorganization Vector (parameters) (Contd....)

Current Row Position	Original Row Position
2	15(Nonreducing sugar)
3	14(Total Carbohydrates)
4	8(Root Weight)
5	5(Fresh Weight)
6	10(Chlorophyll a)
7	6(Seed Weight)
8	13(Reducing sugar)
9	2(Shoot Length)

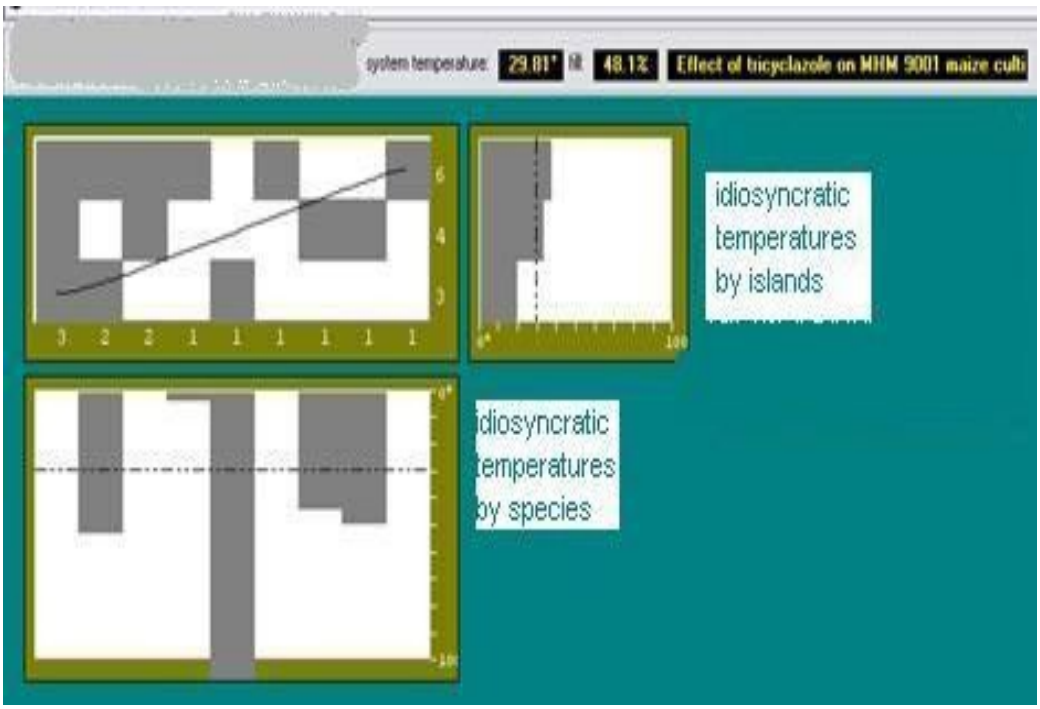

Fig 1. Nestedness pattern with maximally packed matrix with system temperature