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RESEARCH NOTE

Health aspects and ideal temperature for germination of peanut seeds[1]

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ABSTRACT – This study was performed to identify the best temperatures for germinating peanut seeds with fungi on a paper substrate. Eleven seed lots from the cultivars IAC-886 and IAC-503 were selected with different levels of fungi. Two of these seed lots with a high rate of *Aspergillus* spp., *Penicillium* sp., and *Rhizopus* sp., as detected by the blotter test method, were used for selecting a fungicide for seed treatment. Considering the active ingredients evaluated, thiram, at the rate of 300 g of commercial product per 100 kg of seeds, was most efficient in controlling fungi, preventing their interference in germination. All the seed lots, treated with this product or not, were subjected to the germination test on rolls of paper at the temperatures of 25 °C, 30 °C, 35 °C, 20-30 °C, 20-35 °C, 25-30 °C, and 25-35 °C. Alternating temperatures of 20-35 °C, 20-30 °C, and 25-30 °C led to higher germination of peanut seeds, treated with fungicides or not. Constant temperatures are not recommended, because they are more favorable to the development of fungi associated with these seeds than to the germination process, thus underestimating the germination potential of the seeds subjected to the test.

Index terms: *Arachis hypogea*, viability, optimal temperature, pathogens, fungicide.

Aspectos sanitários e temperatura ideal para germinação de sementes de amendoim

RESUMO – Com o objetivo de identificar temperaturas adequadas à germinação de sementes de amendoim com incidência de fungos, em substrato de papel, selecionaram-se onze lotes das cultivares IAC-886 e IAC-503, com diferentes características de qualidade sanitária. Dentre estes, dois com maior incidência de fungos dos gêneros *Aspergillus, Penicillium* e *Rhizopus*, detectados por meio do método do papel de filtro, foram escolhidos para a seleção de um fungicida para o tratamento das sementes. Dentre os princípios ativos avaliados, thiram, na dose de 300 g do produto comercial por 100 kg de sementes, foi o mais eficiente no controle dos fungos, evitando que interferisssem na germinação. Sementes de todos os lotes, tratadas ou não com este produto, foram submetidas ao teste de germinação em rolos de papel, em temperaturas de 25 ºC, 30 ºC, 35 ºC, 20-30 ºC, 20-35 ºC, 25-30 ºC e 25-35 ºC. Concluiu-se que temperaturas alternadas de 20-35 ºC, 20-30 ºC e 25-30 ºC proporcionam maior germinação de sementes de amendoim, tratadas ou não com fungicidas, e que temperaturas constantes não são recomendadas, por serem mais favoráveis ao desenvolvimento de fungos associados às sementes do que ao processo de germinação, subestimando o potencial de germinação das sementes submetidas ao teste.

Termos para indexação: *Arachis hypogea*, viabilidade, temperatura ótima, patógenos, fungicida.

Introduction

Brazil produced 513.5 thousand metric tons of peanut in the 2017/2018 crop season and 94.7% of this production was in the state of São Paulo, where growing this legume crop is an excellent option during the period between sugarcane crops. It promotes recovery of the soil through nitrogen fixation. From 60% to 70% of runner peanut from São Paulo is exported to

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European countries (CONAB, 2018).

Peanut producing companies in São Paulo are also seed producers, and a difficulty faced by the sector is related to the germination test, performed with the aim of receiving the Seed Analysis Certificate for purposes of seed sale. Germination test results obtained in the laboratory are often lower than seedling emergence in the field.

Peanut seed germination procedures in the Rules for Seed Testing foresees the use of two types of substrate, a roll of paper and sand. Temperatures prescribed are those alternating of 20-30 °C and the constant temperatures of 25 °C and 30 °C (Brasil, 2009b). These temperature regimes, however, might not be ideal. Mohamed et al. (1988) registered optimum temperatures from 29 ºC to 36.5 ºC for germination of these seeds, and Nogueira and Távora (2005) found optimum temperatures from 32 ºC to 34 ºC.

Sand is used in the place of paper as a germination substrate when evaluation cannot be practiced through excess of infection, a situation frequently observed in peanut seeds. The problem is so serious that, for this species, according to the Rules for Seed Testing (Brasil, 2009b), seeds may be treated with fungicide in order to carry out the germination test. However, no active ingredient is mentioned for this purpose in this publication.

Infection by fungi, especially of the genera *Aspergillus, Penicillium, Rhizopus,* and *Fusarium* (Santos et al., 2013; Santos et al., 2016), reduces the germination of these seeds upon causing loss of integrity of the cell membranes (Ahamed et al., 2017), a decrease in carbohydrate contents, reducing sugars, proteins, and oil, and an increase in moisture and free fatty acid contents (Kakde and Chavan, 2011; Begum et al., 2013b). Sugars and proteins are used as a growth substrate by such fungi (Chavan, 2011) and the production of lipases, enzymes that catalyze lipid and glyceride hydrolysis in free fatty acids and glycerol, were also reported (Kakde and Chavan, 2011; Chavan, 2011; Manoorkar and Gachande, 2015).

The recommendation for use of sand in this situation is due to the fact that seeds remain more distant from each other than on paper, preventing contamination of seedlings by these fungi. In contrast, when the choice is made to use boxes of sand in the place of paper, considering the procedures adopted in the Rules for Seed Testing (Brasil, 2009b), the method becomes more labor intensive regarding handling, sowing, the greater size and weight of the boxes, the need for standardizing the size of the sand particles, sterilization of the sand before use, and the need for more space, as well as greater difficulty in observing the root system of the seedlings.

In addition to the health problems arising from contamination by fungi, peanut seeds develop in pods under the soil surface and contain high mean oil (31%) and protein (48%) content. For that reason, low physiological potential is common, also due to biochemical changes that reduce lipid stability (Canavar, 2015).

In the state of São Paulo, seeds are analyzed after the fungicide treatment, performed by production companies, since this has been considered an essential practice (Santos et al., 2013) for peanut. Even so, fungal proliferation is common at levels harmful to evaluation of the germination test. There may be temperatures more favorable to seed germination than to fungal development.

Considering the greater practicality of carrying out the germination test on paper than in sand, the aim of this study was to identify temperature regimes more appropriate for conducting this test on paper, associated with treatment of peanut seeds with fungicide or not.

Materials and Methods

The study was performed in the Seed Analysis Laboratory of the Grain and Fiber Center of the *Instituto Agronômico de Campinas* (IAC), in Campinas, SP, Brazil.

Lot selection: six seed lots of the peanut seed cultivar IAC-886 (L1 to L6) were selected and five of the cultivar IAC-503 (L7 to L11). They were manually hulled and had different health quality characteristics, evaluated according to the blotter test method (Brasil, 2009a), conducted with five replications of 20 seeds per lot, placed in Petri dishes (10 seeds per dish) over three sheets of blotter paper, moistened with distilled water, and incubated for seven days at 20±2 °C and a 12-hour photoperiod. Evaluation of fungal structures that developed in the seeds was carried out with the use of a stereoscopic microscope, and confirmation of the genera with the assistance of an optical microscope (Barnett and Hunter, 1999). The results were expressed in percentage per pathogen detected.

Choice of fungicide: seeds from two lots with greater incidence of fungi were used to choose a fungicide effective in control of the pathogens found. The seeds were placed under treatments that consisted of the fungicides generally used by peanut seed producers (rates of the active ingredients and of the commercial products are shown in Table 1), a control without fungicide, and sodium hypochlorite (NaClO) at 1% for 3 minutes. After 24 hours, the health test was set up by the blotter test method, as described above, and the germination test, with four replications of 25 seeds per treatment distributed on rolls of paper toweling moistened with a volume of water equivalent to two and a half times the weight of the dry substrate. This material was kept in a germinator regulated to 30 ºC. The percentages of normal

Table 1. Products and application rates used in treatment of peanut seeds.

Fungicide		Formulation	Application rate of the fungicide.100 kg[1] of seed	
Common name	Commercial name		a.i. (g)(1)	c.p.(2)
Fludioxonil + metalaxyl	Maxim XL®	CS(3)	7.5 + 3,0	300 mL
Carbendazim + thiram	Derosal plus	CS(3)	30 + 70	200 mL
Carboxin + thiram	Vitavax + Thiram	CS(3)	70 + 70	350 mL
Thiram	Mayran	DP(4)	210	300 g

(1) a.i.: active ingredient; (2) c.p.: commercial product; (3) CS: concentrated suspension; (4) DP: dry powder.

seedlings, abnormal and infected seedlings, and of dormant and dead seeds were computed at five and at ten days after sowing, following the criteria of the Rules for Seed Testing (Brasil, 2009b).

Germination temperatures: seeds from the eleven lots were subjected to the germination test, conducted and evaluated as already described, at the constant temperatures of 25 ºC, 30 ºC, and 35 ºC and alternating temperatures of 20-30 ºC, 20-35 ºC, 25-30 ºC, and 25-35 ºC (the higher temperature provided for 8 hours and the lower for 16 hours in each 24-hour cycle), under continuous white fluorescent light, with four replications of 25 seeds per lot, treated (or not) with the fungicide selected in the previous step, Mayran (thiram), at the rate of 300 g of commercial product per 100 kg of seed.

Data analysis: the data obtained in the germination and health tests were subjected to analysis of variance after transformation in ARC SEN $(X/100)^{1/2}$ or in $(X+0.5)^{1/2}$. A completely randomized experimental design was adopted. Data analyses were made separately per cultivar, and for comparison of temperatures, a factorial arrangement (lots × treatments × temperature regimes) was adopted. Mean values were compared by the Scott-Knott test at 5% probability using the SISVAR program (Ferreira, 2011).

Results and Discussion

Fungi from storage of the genera *Aspergillus*, *Penicillium*, and *Rhizopus* were found in seeds of all the lots evaluated. Those of L4 ('IAC-886') and of L7 ('IAC 503') showed the highest incidences of *Rhizopus* sp. (78%) and *Penicillium* sp. (78%), respectively, and L7 was also characterized by seeds with high incidence of *Aspergillus* spp. (76%), and these seed lots were chosen to carry out the fungicide test (Table 2).

Among the active ingredients evaluated, fludioxonil + metalaxyl, carbendazim + thiram, and thiram, in general, reduced the incidence of *Aspergillus* spp., *Penicillium* sp., and *Rhizopus* sp. in the seeds of the two cultivars (Table 3) and even without having completely eliminated the fungi, they favored the growth of normal seedlings in comparison to the untreated control (Table 4), confirming the results obtained by Marchi et al. (2011) in which the treatment with fludioxonil + metalaxyl (100 mL commercial product / 100 kg of seed) resulted in greater physiological potential of the 'IAC 886' seeds, shown in the tests of germination, accelerated aging, and seedling emergence in the field, due to efficient control of *Aspergillus* spp. and *Rhizopus* spp.

Santos et al. (2013) found a reduction in the incidence of *Aspergillus* spp., *Penicillium* spp., and *Rhizopus* sp. in the peanut seed cultivars 'IAC 886' and 'IAC 503', treated with carbendazim + thiram at the same application rate used in the present study, or thiram at a lower rate (100 g commercial product / 100 kg of seed), which provided better results for germination, seedling emergence in sand, and accelerated aging in two consecutive years. The treatment with carboxin + thiram also controlled *Aspergillus* spp. and *Penicillium* sp. in the seeds of 'IAC 886' (Table 3), just as found by Santos

Table 2. Mean initial values (%) of the incidence of fungi in peanut seeds.

Cultivar	Lot	Fungus		
		Aspergillus spp.	*Penicillium* sp.	*Rhizopus* sp.
IAC 886	L1	46 B*	06 C	15 C
	L2	82 A	47 B	01 D
	L3	91 A	40 B	13 C
	L4	09 C	03 C	78 A
	L5	18 C	13 C	16 C
	L6	14 C	18 C	25 C
IAC 503	L7	76 A	78 A	43 B
	L8	65 B	69 A	53 B
	L9	24 C	22 C	60 B
	L10	46 B	36 B	55 B
	L11	13 C	10 C	46 B
CV (%)		26.1	34.3	32.1

*Mean values followed by the same letter in the column do not differ from each other at 5% probability by the Scott-Knott test. Data transformed in ARC SEN $(X/100)^{1/2}$.

et al. (2013). However, it did not reduce the incidence of *Rhizopus* sp. in these seeds, nor of the fungi associated with the cultivar IAC 503 (Table 3); little or no benefit was provided to germination by this product (Table 4). The inefficacy of this fungicide in control of *Rhizopus* in peanut seeds was also found by Bittencourt et al. (2007), regardless of the application rate studied.

Treatment of seeds with sodium hypochlorite reduces contamination without eliminating the fungi present in the inner tissues of the seeds during germination on a paper substrate (Zorato et al., 2001), acting against all types of bacteria, fungi, and viruses by oxidizing biological molecules

Table 3. Mean values (%) of incidence of fungi in peanut seeds in accordance with chemical treatment.

Cultivar	Lot	Treatment	Fungus		
			Aspergillus spp.	*Penicillium* sp.	*Rhizopus* sp.
IAC 886	L4	Fludioxonil + metalaxyl	01 C*	00 C	10 C
		Carbendazim + thiram	00 C	00 C	01 D
		Carboxin + thiram	00 C	01 C	91 A
		Thiram	00 C	00 C	01 D
		NaClO	30 B	13 B	56 B
		Control	61 A	67 A	33 B
CV (%)			50.3	34.8	29.8
IAC-503	L7	Fludioxonil + metalaxyl	01 B	00 B	00 B
		Carbendazim + thiram	01 B	00 B	08 B
		Carboxin + thiram	21 A	25 A	55 A
		Thiram	01 B	00 B	00 B
		NaClO	23 A	21 A	65 A
		Control	07 B	03 B	72 A
CV (%)			50.6	38.6	44.1

*Mean values followed by the same letter in the column do not differ from each other at 5% probability by the Scott-Knott test. Data transformed in $(X+0.5)^{1/2}$.

Table 4. Mean values (%) of normal seedlings (NS), abnormal seedlings (AS), and infected seedlings (IS) and of dormant seeds (DS) and dead seeds (DDS), obtained in the germination test of peanut seeds in accordance with chemical treatment.

Cultivar	Lot	Treatment	Germination Test				
			NS	AS	IS	DS	DDS
IAC 886	L4	Fludioxonil + metalaxyl	26 B*	01	73 B	00	00
		Carbendazim + thiram	16 C	00	84 A	00	00
		Carboxin + thiram	05 C	00	95 A	00	00
		Thiram	56 A	00	44 C	00	00
		NaClO	11 C	00	89 A	00	00
		Control	06 C	00	94 A	00	00
CV (%)			40.3	-	13.8	-	-
IAC 503	L7	Fludioxonil + metalaxyl	50 A	00	50 C	00	00
		Carbendazim + thiram	58 A	00	41 C	01	00
		Carboxin + thiram	36 B	00	64 B	00	00
		Thiram	72 A	00	28 C	00	00
		NaClO	11 C	00	89 A	00	00
		Control	19 C	00	81 A	00	00
CV (%)			18.6	-	13.9	-	-

*Mean values followed by the same letter in the column do not differ from each other at 5% probability by the Scott-Knott test. Data transformed in ARC SEN $(X/100)^{1/2}$.

such as proteins and nucleic acids (Bloomfield et al., 1991). However, a consistent result of the treatment at 1% for 3 minutes was not obtained, which is included in the Rules for Seed Testing (Brasil, 2009b) for control of the fungi associated with peanut seeds (Table 3). The result in this study is also in disagreement with the results reported by Araújo et al. (2004), in which the treatment with NaClO reduced recovery of fungi of the genera *Rhizopus*, *Aspergillus*, *Penicillium*, and *Cladosporium*, regardless of the application rate and immersion time of the seeds. In contrast, Al-Amodi (2016) observed a greater percentage of seed germination for the period of only 6 minutes of immersion.

In addition, it was found during evaluation of germination (Table 4) that *Rhyzopus* sp., when present, spread throughout the roll of paper, contaminating most of the seeds, interfering in the results and reinforcing the need for control of its development in this test. This fact corroborated the observations of Moraes and Mariotto (1985) performed in health tests where, due to rapid growth, *Rhizopus* spp. covered the Petri dish, hindering or impeding visualization of other microorganisms associated with the seeds.

The dissemination of *Aspergillus* spp. and of *Penicillium* ssp. among the seeds on the germination paper was also observed, probably due to the humid microclimate having favored the development of these fungi, promoting contamination. This also hurt evaluation of the test due to absence of treatment (control) or of ineffectiveness of fungicide effect (Table 3), shown in the incidence of infected seedlings (Table 4). When not controlled, fungi of the genus *Aspergillus* produce aflatoxins, which inhibit incorporation of amino acids to proteins and synthesis of amylase (Janardhan et al., 2011), impeding seed germination or elongation of the hypocotyl and of the roots of the seedlings (Begum et al., 2013a; Al-Amodi, 2015). *Penicillium* spp. cause rotting of seeds and serious lesions in seedlings (Ito et al., 1992) since they exhibit greater activity of lipase, an enzyme that degrades lipids, from among the fungi isolated from abnormal peanut seedlings (Kakde and Chavan, 2011).

Among the fungicides most efficient in control of fungi associated with the seeds (Tables 3 and 4), thiram provided higher percentages of germination as a result of lower occurrence of infected seedlings (Table 4), probably due to the wide range of action against fungi detected in the outer part of the seeds, which may also have contributed to reduce dissemination by the paper. Furthermore, due to the mode of action by contact, its active ingredient is little absorbed by the seed and shows little translocation within the seedlings, a fact that may also have led to low phytotoxicity.

The other fungicides are mixtures of active ingredients of contact, fludioxonil or thiram, with active ingredients of systemic action, metalaxyl, carbendazim, or carboxin, of broad spectrum, which penetrate the seed coat and translocate to seedling parts in development during germination. This could increase efficiency in control of the fungi. However, this was not confirmed, and the application rate of thiram of 70 g a.i. / 100 kg of seed, combined with carbendazim or carboxin, one third of the thiram applied in isolation, of 210 g a.i. / 100 kg of seed, may also have resulted in lower protection of the seeds. Furthermore, as in the roll of paper, the seeds remained near one another, and the same leaching of the fungicide is not found as occurs in the soil, there is greater absorption of the active ingredient, with a greater chance of phytotoxicity caused by a product with systemic action. Intoxicated seedlings may have been more susceptible to the infection by remaining fungi located further within the seeds.

Thus, thiram was chosen for treatment of the seeds that underwent the study of temperatures in germination, with the purpose of eliminating or reducing interference of the fungi in the test conducted on paper and of allowing comparison with results obtained without the treatment.

Optimum temperature is what allows the most efficient combination between germination percentage and speed, providing for more regular, rapid, and complete germination of seed samples of a determined species (Marcos-Filho, 2015; Brasil, 2009b). Nevertheless, alternating temperatures provided greater effectiveness in the germination process of peanut seeds than constant temperatures, and the alternating 20-35 ºC temperatures stood out (Table 5). This combination of temperatures promoted the maximum values of germination achieved for most of the seed lots not treated with fungicide, due to the lower incidence, in general, of infected seedlings (Table 6), abnormal seedlings (Table 7), and dead seeds (Table 8). Constant temperatures mainly caused greater seedling infection (Table 6); and the temperature of 35 ºC also increased the percentage of dead seeds, compatible with that obtained unexpectedly at 20-30 ºC (Table 8).

In treated seeds, fungal control provided by the fungicide resulted in reduction of the differences in the values of normal seedlings (Table 5) observed among differing temperatures in the untreated seeds, such that for L3, L8, and L10, there were no significant differences among the temperature regimes studied. Nevertheless, the alternating temperatures (except for 25-35 ºC) proved to be more adequate since they led to the highest values of germination for most of the lots (Table 5), due to the lower propensity to seedling infection (Table 6). Although 25-35 ºC provided indexes of infected seedlings (Table 7) in a general way similar to that of the other alternating temperatures, such an advantage was not reflected

in higher germination values (Table 5), due to the greater occurrence of abnormal seedlings (Table 7). For L1, L2, L3, L8, and L11, there was also greater occurrence of dead seeds (Table 8) in this combination of temperatures.

It should be emphasized that the treatment with thiram visibly increased the values of seed lots germination potential, regardless of the temperature regime adopted for germination, reaffirming the effectiveness of this product in treatment of peanut seeds (Table 5).

The requirement for alternating temperatures for germination is generally associated with dormancy. The reasons for such a requirement are not fully known; it is supposed that thermal variation alters the balance between germination promotors and inhibitors so that the concentration of inhibitors decreases in the period of lower temperature and that of the promotors increases at the higher temperature (Marcos-Filho, 2015). In peanut seeds, dormancy is overcome during storage as the concentration of ABA decreases and that of cytokinin increases (Narasimhareddy and Swamy, 1979). However, even after six months of storage and with a nearly

Table 5. Mean values (%) of normal seedlings obtained in the germination test of peanut seeds with and without fungicide treatment (FT) with thiram, at the rate of 300 g of commercial product per 100 kg of seed.

Cultivar	Lot	FT	Temperature Regime						
			25 ºC	30 ºC	35 ºC	20-30 ºC	20-35 ºC	25-30 ºC	25-35 ºC
IAC 886	L1	Without	02 Cc*	19 Bb	18 Ab	43 Ba	29 Bb	46 Aa	36 Aa
	L2		56 Aa	47 Ab	35 Ab	71 Aa	57 Aa	40 Ab	46 Ab
	L3		05 Cc	24 Bb	22 Ab	33 Bb	69 Aa	51 Aa	25 Ab
	L4		05 Cb	06 Cb	03 Bb	28 Ba	40 Ba	18 Ba	32 Aa
	L5		20 Bd	28 Ac	15 Bd	55 Ab	75 Aa	35 Ac	41 Ac
	L6		23 Bc	40 Ab	10 Bc	41 Bb	66 Aa	40 Ab	18 Ac
	L1	With	84 Ba	70 Aa	47 Bb	79 Ba	83 Aa	84 Ba	68 Ba
	L2		69 Ba	66 Aa	39 Bb	67 Ba	60 Ba	73 Ba	62 Ba
	L3		74 Ba	66 Aa	57 Ba	80 Ba	81 Aa	82 Ba	73 Ba
	L4		77 Bb	56 Ac	85 Ab	79 Bb	84 Ab	95 Aa	91 Aa
	L5		89 Ab	83 Ab	78 Ab	96 Aa	94 Aa	98 Aa	80 Ab
	L6		94 Aa	65 Ab	83 Aa	93 Aa	84 Aa	90 Ba	82 Aa
CV (%)			18.4						
Cultivar	Lot	FT	Temperature Regime						
			25 ºC	30 ºC	35 ºC	20-30 ºC	20-35 ºC	25-30 ºC	25-35 ºC
IAC 503	L7	Without	06 Ab	19 Ab	12 Ab	44 Aa	33 Ba	37 Aa	37 Aa
	L8		14 Ac	18 Ac	15 Ac	29 Ab	33 Bb	55 Aa	41 Ab
	L9		18 Ab	24 Ab	06 Bc	25 Ab	60 Aa	24 Bb	26 Bb
	L10		10 Ab	04 Bc	18 Ab	43 Aa	53 Aa	48 Aa	50 Aa
	L11		06 Ab	14 Aa	05 Bb	07 Bb	26 Ba	08 Cb	22 Ba
	L7	With	72 Bb	72 Ab	78 Ab	88 Aa	89 Aa	76 Bb	78 Ab
	L8		79 Ba	73 Aa	68 Aa	75 Aa	71 Ba	71 Ba	59 Ba
	L9		89 Aa	75 Ab	79 Ab	91 Aa	92 Aa	91 Aa	81 Ab
	L10		78 Ba	73 Aa	75 Aa	85 Aa	87 Aa	91 Aa	80 Aa
	L11		74 Bb	57 Aa	71 Aa	84 Aa	85 Aa	86 Aa	60 Bb
CV (%)			17.9						

*Mean values followed by the same uppercase letter in the column and lowercase letter in the row do not differ from each other at 5% probability by the Scott-Knott test. Data transformed in ARC SEN $(X/100)^{1/2}$.

null incidence of dormant seeds (data not shown), alternating temperatures favored germination.

The temperature regimes of 20-35 ºC and 20-30 ºC, followed by the regimes of 25-30 ºC and 25-35 ºC, also resulted, in general, in lower incidences of infected seedlings (Table 6) arising from untreated seeds, confirming the suitability of these temperatures. Upon favoring seed germination, they decreased susceptibility of the seeds to the action of microorganisms.

In addition, alternating temperatures were probably less favorable to fungal growth, resulting in lower seedling infection (Table 6). At 30 ºC and at 35 ºC, there was development of *Rhizopus* sp. throughout the roll that contained seeds in germination, confirming the report of Moraes and Mariotto (1985) regarding the speed of growth of this fungus. Temperatures of 35 ºC, 40 ºC, and 42 ºC have been related to optimal growth of the fungi of this genus (Han et al., 2003). However, in the alternating temperature regimes, the supply of 30 ºC or 35 ºC was interrupted for 16 hours with lower temperatures, which probably also favored germination of the seeds in relation to the growth of these fungi.

Table 6. Mean values (%) of infected seedlings obtained in the germination test of peanut seeds with and without fungicide treatment (FT) with thiram at the rate of 300 g of the commercial product per 100 kg of seed.

Cultivar	Lot	FT	Temperature Regime						
			25 ºC	30 ºC	35 ºC	20-30 ºC	20-35 ºC	25-30 ºC	25-35 ºC
IAC 886	L1	Without	97 Aa*	81 Ab	65 Ab	50 Ab	70 Ab	53 Bb	63 Ab
	L2		43 Ca	53 Ba	61 Aa	29 Aa	42 Ba	56 Ba	51 Aa
	L3		95 Aa	76 Ab	64 Ac	53 Ac	30 Bd	49 Bc	75 Ab
	L4		95 Aa	94 Aa	80 Ab	40 Ad	60 Ac	82 Ab	68 Ac
	L5		80 Ba	72 Ba	81 Aa	40 Ab	24 Bb	65 Ba	59 Aa
	L6		77 Ba	59 Bb	78 Aa	54 Ab	32 Bc	58 Bb	82 Aa
	L1	With	06 Bb	30 Aa	42 Aa	13 Ab	14 Bb	12 Ab	15 Ab
	L2		30 Aa	34 Aa	47 Aa	30 Aa	38 Aa	23 Aa	21 Aa
	L3		18 Ab	30 Aa	42 Aa	17 Ab	10 Bb	13 Ab	10 Ab
	L4		21 Ab	44 Aa	14 Bb	19 Ab	13 Bb	04 Bc	02 Ac
	L5		07 Bb	15 Aa	18 Ba	03 Bb	01 Bb	00 Bb	04 Ab
	L6		03 Bb	35 Aa	14 Bb	04 Bb	13 Bb	09 Ab	09 Ab
CV (%)			26.6						
Cultivar	Lot	FT	Temperature Regime						
			25 ºC	30 ºC	35 ºC	20-30 ºC	20-35 ºC	25-30 ºC	25-35 ºC
IAC 503	L7	Without	93 Aa	81 Ba	72 Ab	53 Ab	67 Ab	60 Cb	62 Bb
	L8		85 Aa	82 Ba	77 Aa	52 Ab	65 Ab	44 Cb	58 Bb
	L9		81 Aa	76 Ba	86 Aa	72 Aa	38 Bb	75 Ba	74 Aa
	L10		89 Ab	96 Aa	76 Ab	46 Ac	47 Bc	52 Cc	49 Bc
	L11		94 Aa	85 Ba	83 Aa	59 Ac	74 Ab	92 Aa	78 Ab
	L7	With	20 Aa	28 Aa	17 Aa	06 Ab	09 Ab	17 Aa	06 Bb
	L8		09 Aa	20 Aa	30 Aa	12 Aa	23 Aa	17 Aa	13 Aa
	L9		09 Ab	24 Aa	15 Aa	08 Ab	07 Ab	09 Ab	01 Bb
	L10		16 Aa	24 Aa	17 Aa	09 Ab	06 Ab	09 Ab	07 Bb
	L11		25 Aa	41 Aa	27 Aa	13 Ab	11 Ab	13 Ab	25 Aa
CV (%)			24.0						

*Mean values followed by the same uppercase letter in the column and lowercase letter in the row do not differ from each other at 5% probability by the Scott-Knott test. Data transformed in ARC SEN $(X/100)^{1/2}$.

The same reasoning can also be applied to the other fungi detected (Table 2), because there is information regarding maximum growth of *Penicillium* at around temperatures of 30 ºC (Pezzini et al., 2005), from 30 to 35 ºC (Francisco and Usberti, 2008), and from 20 to 30 ºC, but with good tolerance for temperatures from 10 to 40 ºC for *Penicillium digitatum* (Carrillo-Inungaray et al., 2014; Minamor and Odamtten, 2017), as well as records of greater activity of lipase at 30 ºC for *Penicillium chryzogenum* (Kakde and Chavan, 2011). For *Aspergillus,* the optimum growing temperatures registered were 30 ºC, (Pezzini et al., 2005), 40 ºC (Dantigny et al., 2005), and 35 ºC (Francisco and Usberti, 2008).

Moreover, the greater the moisture content, from 10.2% to 18.5%, in common bean seeds in storage, the greater the incidence of *Aspergillus* spp. and *Penicillium* spp. (Francisco and Usberti, 2008). For that reason, it is evident that in the humid microclimate, characteristic of the germination test, water was not a limiting factor to development of the fungi detected in peanut seeds.

This study confirmed that the fungal microflora can in fact disguise results of maximum percentage of normal seedlings that can be obtained from peanut seed lots provided by the germination test. From this, it can be inferred that the optimal conditions for conducting this test for seeds of this species must be defined as those that result in the best combination between seed germination percentage and the fungal growth associated with it.

Table 7. Mean values (%) of abnormal seedlings obtained in the germination test of peanut seeds with and without fungicide treatment (FT) with thiram at the rate of 300 g of the commercial product per 100 kg of seed.

Cultivar	Lot	FT	Temperature Regime						
			25 ºC	30 ºC	35 ºC	20-30 ºC	20-35 ºC	25-30 ºC	25-35 ºC
IAC 886	L1	Without	00 Aa*	00 Aa	00 Aa	00 Aa	01 Aa	01 Aa	00 Aa
	L2		00 Aa	00 Aa	00 Aa	00 Aa	00 Aa	01 Aa	03 Aa
	L3		00 Aa	00 Aa	02 Aa	00 Aa	01 Aa	00 Aa	00 Aa
	L4		00 Aa	00 Aa	00 Aa	00 Aa	00 Aa	00 Aa	00 Aa
	L5		00 Aa	00 Aa	00 Aa	01 Aa	01 Aa	00 Aa	00 Aa
	L6		00 Aa	01 Aa	00 Aa	00 Aa	02 Aa	02 Aa	00 Aa
	L1	With	09 Aa	00 Ac	04 Ac	05 Ab	01 Ac	04 Ab	12 Aa
	L2		01 Bb	00 Ab	03 Ab	01 Ab	00 Ab	03 Ab	13 Aa
	L3		08 Aa	01 Ab	01 Ab	01 Ab	03 Ab	03 Ab	07 Ba
	L4		01 Ba	00 Aa	01 Aa	01 Aa	03 Aa	01 Aa	06 Ba
	L5		04 Bb	02 Ab	03 Ab	01 Ab	03 Ab	02 Ab	14 Aa
	L6		03 Bb	00 Ab	03 Ab	03 Ab	03 Ab	01 Ab	08 Ba
CV (%)			62.5						
Cultivar	Lot	FT	Temperature Regime						
			25 ºC	30 ºC	35 ºC	20-30 ºC	20-35 ºC	25-30 ºC	25-35 ºC
IAC 503	L7	Without	00 Aa*	00 Aa	00 Aa	00 Ba	01 Aa	01 Aa	00 Aa
	L8		00 Aa	00 Aa	00 Aa	00 Ba	02 Aa	03 Aa	01 Aa
	L9		00 Aa	00 Aa	00 Aa	00 Ba	02 Aa	01 Aa	00 Aa
	L10		00 Aa	00 Aa	00 Aa	00 Ba	00 Aa	00 Aa	01 Aa
	L11		00 Ab	00 Ab	00 Ab	20 Aa	00 Ab	00 Ab	00 Ab
	L7	With	05 Ba	00 Bb	05 Aa	02 Bb	01 Ab	04 Ba	11 Aa
	L8		09 Aa	05 Aa	00 Bb	08 Aa	01 Ab	10 Aa	15 Aa
	L9		02 Bb	00 Bb	05 Ab	01 Bb	01 Ab	00 Cb	17 Aa
	L10		06 Aa	03 Ab	07 Aa	05 Aa	04 Ab	00 Cb	09 Aa
	L11		01 Bb	02 Ab	01 Bb	02 Bb	04 Aa	01 Cb	08 Aa
CV (%)			62.7						

*Mean values followed by the same uppercase letter in the column and lowercase letter in the row do not differ from each other at 5% probability by the Scott-Knott test. Data transformed in $(X+0.5)^{1/2}$.

Table 8. Mean values (%) of dead seeds obtained in the germination test of peanut seeds with and without fungicide treatment (FT) with thiram at the rate of 300 g of the commercial product per 100 kg of seed.

Cultivar	Lot	FT	Temperature Regime						
			25 ºC	30 ºC	35 ºC	20-30 ºC	20-35 ºC	25-30 ºC	25-35 ºC
IAC 886	L1	Without	01 Ac*	00 Ac	17 Aa	07 Bb	00 Ac	00 Ac	01 Ac
	L2		01 Aa	00 Aa	04 Ba	00 Ca	01 Aa	03 Aa	00 Aa
	L3		00 Ab	00 Ab	12 Aa	14 Aa	00 Ab	00 Ab	00 Ab
	L4		00 Ab	00 Ab	17 Aa	32 Aa	00 Ab	00 Ab	00 Ab
	L5		00 Ab	00 Ab	04 Ba	04 Ba	00 Ab	00 Ab	00 Ab
	L6		00 Ac	00 Ac	12 Aa	05 Bb	00 Ac	00 Ac	00 Ac
	L1	With	01 Ab	00 Ab	07 Aa	03 Aa	02 Ab	00 Ab	05 Ba
	L2		00 Ab	00 Ab	11 Aa	02 Ab	02 Ab	01 Ab	04 Ba
	L3		00 Ab	03 Ab	00 Bb	02 Ab	06 Aa	02 Ab	10 Aa
	L4		01 Aa	00 Aa	00 Ba	01 Aa	00 Aa	00 Aa	01 Ba
	L5		00 Aa	00 Aa	01 Ba	00 Aa	02 Aa	00 Aa	02 Ba
	L6		00 Aa	00 Aa	00 Ba	00 Aa	00 Aa	00 Aa	01 Ba
CV (%)			67.2						
Cultivar	Lot	FT	Temperature Regime						
			25 ºC	30 ºC	35 ºC	20-30 ºC	20-35 ºC	25-30 ºC	25-35 ºC
IAC 503	L7	Without	01 Ab	00 Ab	16 Aa	03 Bb	00 Ab	00 Ab	01 Ab
	L8		01 Ac	00 Ac	08 Bb	19 Aa	00 Ac	00 Ac	00 Ac
	L9		01 Ac	00 Ac	08 Ba	03 Bb	00 Ac	00 Ac	00 Ac
	L10		01 Ac	00 Ac	06 Bb	11 Aa	00 Ac	00 Ac	00 Ac
	L11		00 Ab	00 Ab	12 Aa	14 Aa	00 Ab	00 Ab	00 Ab
	L7	With	03 Aa	00 Ab	00 Ab	04 Aa	01 Bb	03 Aa	05 Ba
	L8		03 Ab	02 Ab	02 Ab	05 Ab	05 Ab	02 Ab	13 Aa
	L9		00 Aa	00 Aa	01 Aa	00 Ba	00 Ba	00 Ba	01 Ca
	L10		00 Ab	00 Ab	01 Ab	01 Bb	03 Aa	00 Bb	04 Ba
	L11		00 Ab	00 Ab	01 Ab	01 Bb	00 Bb	00 Bb	07 Aa
CV (%)			50.0						

*Mean values followed by the same uppercase letter in the column and lowercase letter in the row do not differ from each other at 5% probability by the Scott-Knott test. Data transformed in $(X+0.5)_{1/2}$.

Conclusions

The temperatures most adequate for germination of peanut seeds in paper substrate are alternating temperatures of 20-35 ºC, followed by those of 20-30 ºC and 25-30 ºC.

Constant temperatures are not recommended, because they are more favorable to the development of fungi associated with these seeds than with the germination process, underestimating the germination potential of the seeds subjected to the test.

Thiram at the application rate of 300 g of commercial product per 100 kg of seed is an option for treatment of peanut seeds when they are subjected to the germination test on paper.

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