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## The Effect of *N,N*-Diethyl-3-methylbenzamide (DEET) on the Germination of *Raphanus sativus* (Radish Plants)

Caimin Xi<sup>1</sup> and James T. Zacharia<sup>2</sup>

<sup>1</sup>Adlai Stevenson High School, Illinois, 60069, USA

<sup>2</sup>Dar es Salaam University College of Education, P. O. Box 2329, Dar es Salaam, Tanzania

### ABSTRACT

DEET is one of the major chemical constituents of bug repellent with an estimated global use of 7 million liters in 2016. While there has been research concerning the health safety of bug repellent, research concerning its environmental impact is very limited. This study was designed to determine the impact bug repellent on the germination of plant seeds. The build-up of bug repellent found in water and soil was simulated by exposing radish seeds to various concentrations of repellent during the germination process. This two-phased experimental set up demonstrated a significant correlation between higher bug repellent concentrations and lower germination rates. Even in the group containing 0.01% concentration of repellent, only 60% of seeds germinated comparing to 93.3% in the control group on day 10. The differences in germination rates was found to be statistically significant ( $P=0.0025$ ). The experimental groups with repellents also delayed the process of germination.

**Keywords:** *N,N*-Diethyl-3-methylbenzamide, DEET, Germination, *Raphanus sativus*, Radish Plants

### \*Correspondence to Author:

James T. Zacharia  
Dar es Salaam University College  
of Education, P. O. Box 2329, Dar  
es Salaam, Tanzania

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## Introduction

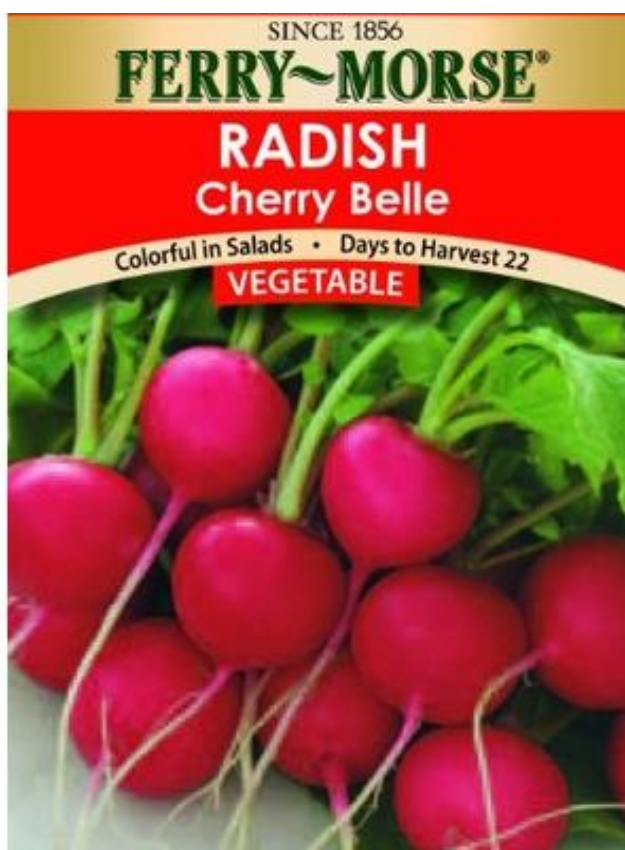
Bug repellent is a common household product that is used in great quantities worldwide. According to U.S. Census data and Simmons National Consumer Survey, approximately 184.78 million Americans in the United States used bug spray in 2016 (NHCS, 2016). Not only is a lot of bug repellent being used, but also a major portion of the repellent is absorbed in the environment. This is because bug repellent is typically in the form of sprays and is used in open area outdoors. Hence only a portion of the repellent will end up on a person's body or clothing; majority of the spray will instead be spread into the atmosphere and absorbed by the environment. Bug repellent can enter the environment through several pathways such as; directly into the atmosphere during spray application; directly into surface of soil or water from overspray; or indirectly via wastewater treatment plant discharges (Weeks J., Guiney P., & Nikiforov A, 2011).

Whether bug repellent is introduced into the environment through direct or indirect ways, the chemicals are building up in water, soil and sediments. This build up in water, soil and

sediments is of extreme concern as it may directly affect the germination and growth of some plants. Moreover, a large portion of bug sprays that is entering the environment uses DEET as its primary active ingredient. In fact, DEET is the most commonly used active ingredient in bug repellents. Although DEET was approved by EPA to be safe for use in bug repellent and other cosmetic products, recently it has been proven that the chemical is not entirely safe for use. In fact, DEET containing bug repellent has been shown to act as a pesticide, harming wildlife and pets alike. Furthermore, the chemical has recently been put into question about its safety towards humans as there has been reported cases showing that it contributes to some severe human illnesses such as behavioral changes, ataxia, encephalopathy, seizures, and comas (Goddard, 2002).

## Materials and Methods

Cherry Belle Radish Seeds, 11 Freezer Zip-Lock Bags, Paper Towels, Tweezers, 10 mL & 20 mL graduated cylinders, Plastic wrap, Tap water, OFF! Deep Woods bug repellent and 1 mL Pipette.



## Experimental Procedures

Two sets of experimental procedures were devised with different levels of repellent concentrations

### Experiment Set 1: Higher Concentrations of Repellent

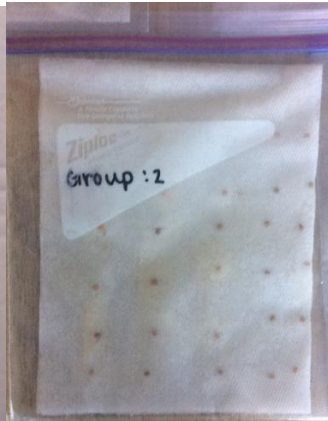
Various amount of bug repellents were measured and placed in 5 different freezer ziploc bags labelled 1 to 5 as follows;

Group 1 (Control): 20 mL tap water only, Group 2 (100%): 20 mL bug repellent, Group 3 (75%): 5 mL water, 15 mL bug repellent, Group 4 (50%): 10 mL water, 10 mL bug repellent, and Group 5 (25%): 15 mL water, 5 mL bug repellent. Each solution was stirred until thoroughly mixed. Then 5 pieces of paper towel each measuring 11" by 7" were prepared followed by 5 pieces of plastic wrap large enough to cover the paper towel. Each of the paper towel were placed horizontally on plastic wrap. 10 mL of solution were poured

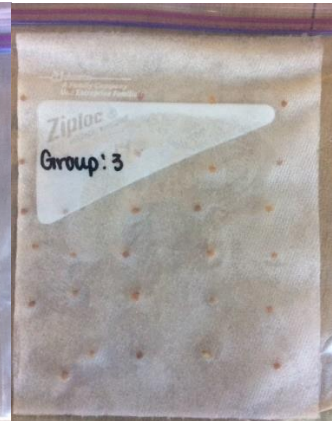
onto the left half of the paper towel to help the seeds stay in place and limit movement. Using tweezers, the 30 radish seeds were evenly distributed in 6 rows each with 5 seeds onto the left half of the paper towel which is already soaked in liquid. The right side of the paper towel was brought over the left half so that the wet and dry portions of the paper towel are now on top of each other. The sides were pressed together so that the liquid is spread throughout both layers of paper towels and then the folded paper towel was placed into the respectively labelled Ziploc Bags. The remaining 10 mL of solution was poured into the bag ensuring it is evenly distributed. The Ziploc bags were tightly sealed and placed in an easily accessible place at room temperature. All other conditions necessary for seed germination were maintained. The seeds were monitored on daily basis for 10 days and observations were record on how many have germinated each day.



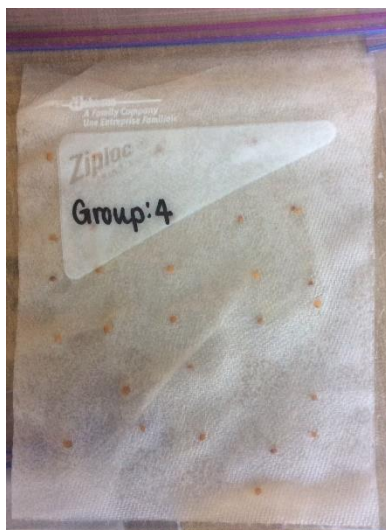
Control Group 1: (0%Rplt)



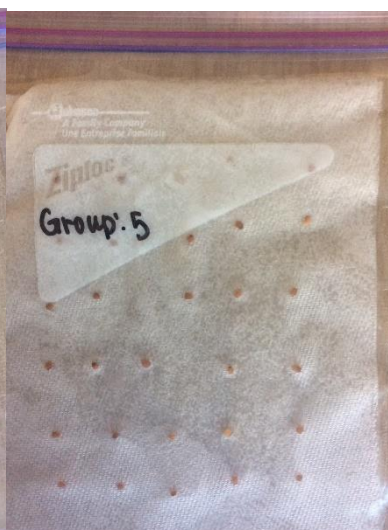
Group 2: 100% Rplt



Group 3: 75% Rplt



Group 4: 50% Rplt



Group 5: 25% Rplt

**Experiment Set 2: Lower Concentrations of Repellant**

For this set of experiment, same experimental procedures were followed as 2.1.1 but this time lower concentrations of repellants were used as shown below; Group 1 (Control): 18 mL tap

Water, 0.0 mL repellent ; Group 2 (10%): 1.8 mL repellent; Group 3 (1%): 0.18 mL repellent ; Group 4 (0.1%): 0.018 mL repellent ; Group 5 (0.01%): 0.0018 mL repellent ; Group 6: (0.001%): 0.00018 mL repellent



Key



U=Ungerminated

C= Cracked

S= Sprouted

L= Leaf

**Results and Discussion**

For experiment set 1, the observation results after 10 days are summarized in table 1.

Experimental groups 2, 3, 4 and 5 containing concentrations varying from 100% to 25% resulted into absolutely no germination after 10 days of observation. In comparison, Control Group 1 had 14 seeds that germinated on day 1, and by day 7 it had already reached the maximum of 27 germinated radish seeds. Since

none of the seeds germinated in all experimental groups in set 1, the assumption here was that higher concentrations of repellants adversely hindered the germination of radish seeds. This observation therefore necessitated to conduct a second experimental set up (set 2). The purpose of experiment set 2 was to identify the lowest level of bug repellent that would have no impact on the germination of radish seeds.

**Table 1: Seed Germination status for experiment set 1 over the study period**

Days	Date	Group 1 Control (0% Rplt)	Group 2 (100% Rplt)	Group 3 (75% Rplt)	Group 4 (50% Rplt)	Group 5 (25% Rplt)
1	11/25/16	14	0	0	0	0
2	11/26/16	18	0	0	0	0
3	11/27/16	22	0	0	0	0
4	11/28/16	24	0	0	0	0
5	11/29/16	25	0	0	0	0
6	11/30/16	25	0	0	0	0
7	12/1/16	27	0	0	0	0
8	12/2/16	27	0	0	0	0
9	12/3/16	27	0	0	0	0
10	12/4/16	27	0	0	0	0

(N=30 per group)

**Table 2: Germination status for experiment set 2 over the study period**

Date	Group 2 (10%)				Group 3 (1%)				Group 4 (0.1%)				Group 5 (0.01%)				Group 6 (0.001%)				Group 1 (control)						
	C	S	L	U	C	S	L	U	C	S	L	U	C	S	L	U	C	S	L	U	C	S	L	U	C	S	L
1/8/2017	0	0	0	30	2	0	0	28	1	0	0	29	7	0	0	23	9	0	0	11	19	0	0	11			
1/9/2017	0	0	0	30	2	0	0	28	6	0	0	24	8	7	0	15	6	15	0	9	5	21	0	4			
1/10/2017	0	0	0	30	2	0	0	28	4	3	0	23	5	4	8	13	4	7	11	8	4	7	16	3			
1/11/2017	0	0	0	30	2	0	0	28	4	3	0	23	5	4	8	13	4	5	13	8	4	6	18	2			
1/12/2017	0	0	0	30	2	0	0	28	5	3	0	22	3	6	8	13	3	6	13	8	3	7	18	2			
1/13/2017	0	0	0	30	2	0	0	28	5	3	0	22	3	5	9	13	2	7	14	7	3	6	19	2			
1/14/2017	0	0	0	30	2	0	0	28	5	3	0	22	3	5	10	12	2	7	15	6	3	6	19	2			
1/15/2017	0	0	0	30	2	0	0	28	6	3	0	21	3	5	10	12	2	6	16	6	3	4	21	2			
1/16/2017	0	0	0	30	2	0	0	28	6	3	0	21	2	5	11	12	2	6	16	6	2	4	22	2			
1/17/2017	0	0	0	30	2	0	0	28	6	3	0	21	2	5	11	12	2	6	16	6	2	3	23	2			

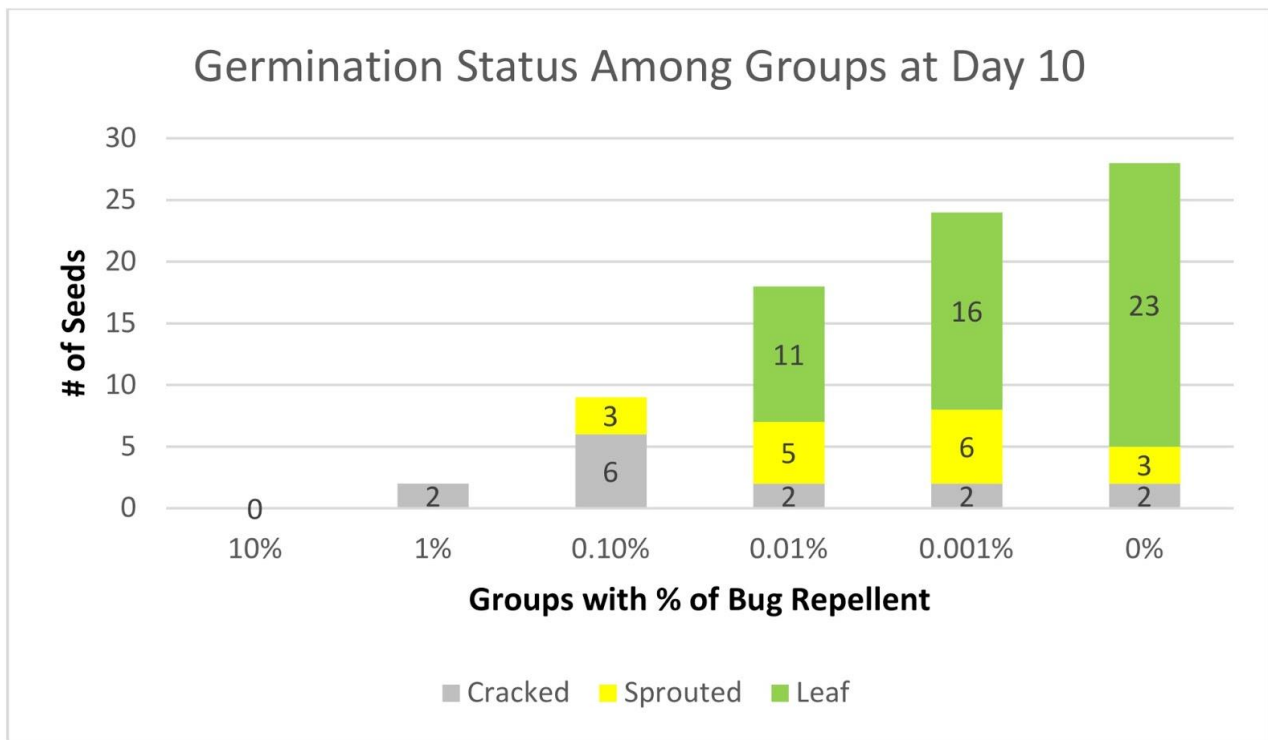
N=30 per each group

The different germination status of radish seeds for experiment set 2 in each group over the 10 days of experimental period was as shown in Table 2. In group 2 containing 10% of bug repellent not even a single radish seed germinated implying that this concentration level

is still higher enough to hinder the germination of seeds. This is similar to the results in experiment set 1 where there was no germination at all concentration levels used in set 1. The concentration of group 2 was diluted 10 times creating group 3 containing 1% of repellent. This

resulted in only 6.6% (2/30) of the seeds reaching the cracking stage of germination. In group 4 containing 0.1% repellent, the germination rate increased to 30% (9/30). Even in group 5 containing 0.01% of repellent, only 60% (18/30) of seeds germinated. The difference between group 5 and control group 1 is statistically significant ( $p=0.0025$ , Chi square test by MedCal Software). The damage bug repellent caused can be further observed in group 6 which contained 0.001% of bug repellent. At the end of 10 days, only 80% (24/30) seeds germinated in group 6. In comparison, 94% (28/30) of the radish seeds germinated in the control group 1.

The speed and stage of growth are important factors for seeds during the germination process. In this study not only does the quantity of seeds that germinated matters, but the time and the extent to which it germinated was also taken into account. These factors are important because, in the natural environment they greatly impact the growth and development of plants. In this experiment, cracked and sprouted seeds were technically considered to be germinated, however in earth's ecosystem these seeds would not have survived. From this perspective, bug repellent has a huge impact on the environment as it prevented the seeds from fully germinating and thus will never become a mature plant.



**Figure 1: Germination status among groups on day 10**

Increased germination rates were observed in groups having lower concentrations of the bug repellents (Figure 1). By day 10, the amount of germinated seeds varied significantly among the 6 groups with concentrations between 10% and 0% of bug repellents (10 times dilution from a higher concentration group). As for the growth stages of germinated seeds, they were split into 3 categories: cracked (C), sprouted (S), and leaf (L). Group 3 with 1% repellent, only 2 seeds

reached the cracked stage and no seeds progressed to a further stage of germination. Similarly, group 4 with 0.1% of repellent concentration 6 seeds cracked and 3 seeds sprouted, but no seeds ever developed any leaves. Groups with concentrations of 0.01% and 0.00%, seeds were able to progress to all stages of germination. Nonetheless the amount of seeds that grew leaves were impacted by the concentration levels. At 0.01% concentration

only 36.7% of seeds grew leaves, while at 0.001% repellent concentration 53.3% of seeds grew leaves, and with 0% repellent 76.7% of radish seeds reached a full stage with leaves.

**Statistical Test**

Chi square statistics test was used to compare the germination rates between experimental groups as shown in table 3.

**Table 3: Statistics Test of the germination rates between groups on day 10**

<u>% Bug Repellant</u> <u>(% Germination)</u>	<u>0% BR</u> <u>(93.3%)</u>	<u>0.001% BR (80%)</u>	<u>0.01% BR</u> <u>(60%)</u>	<u>0.1% BR</u> <u>(30%)</u>	<u>1% BR</u> <u>(6.67%)</u>	<u>10% BR</u> <u>(0%)</u>
<u>0% BR</u> <u>(93.3%)</u>						
<u>0.001% BR</u> <u>(80%)</u>	$\chi^2 = 2.26$ $p = 0.133$					
<u>0.01% BR</u> <u>(60%)</u>	$\chi^2 = 9.136$ $p = 0.0025$	$\chi^2 = 2.81$ $p = 0.0937$				
<u>0.1% BR</u> <u>(30%)</u>	$\chi^2 = 24.998$ $p < 0.001$	$\chi^2 = 14.899$ $p = 0.0001$	$\chi^2 = 5.364$ $p = 0.0206$			
<u>1% BR</u> <u>(6.67%)</u>	$\chi^2 = 44.278$ $p < 0.001$	$\chi^2 = 32.300$ $p < 0.001$	$\chi^2 = 18.877$ $p < 0.001$	$\chi^2 = 5.362$ $p = 0.0206$		
<u>10% BR</u> <u>(0%)</u>	$\chi^2 = 51.590$ $p < 0.001$	$\chi^2 = 39.333$ $p < 0.0001$	$\chi^2 = 25.286$ $p < 0.0001$	$\chi^2 = 10.412$ $p = 0.0013$	$\chi^2 = 2.036$ $p = 0.1537$	

Table 3 above shows the Chi Square Statistical test to compare the germination percentage between the groups on day 10. The *p* value represents significance level and shows the chance that the results were solely by chance. The closer to 0, the more significant the results were and less chance the results were a mistake. For this experiment, every *p* value under 0.05 is considered significant. All comparisons excluding three, were considered significant, proving the difference of the germination percentage among groups is not by random but rather they were altered by the varying concentrations of bug repellent.

The impact of the repellent cannot only be seen on the final experimental germination day 10, but can also be demonstrated as early as day 1 as

shown in Figure 2. After only 24 hours of exposure to the solutions containing repellants, the highest germination group for any of the repellent exposure group is 30% (9/30 in Group 6, 0.001%). It is statistically different from the control group where 63.3% (19/30) were germinated in day 1 (P=0.01). The differences of total germinated seeds was maintained between groups over the entire study period. Figure 2 also shows that there is a delay in the seeds germination in groups treated with repellent solutions, such that on day 1, 67.8% (19/28) of the seeds had already germinated in the control group. However, even in Group 6 containing 0.01% repellent, only 37.5% (9/24) of the seeds that eventually germinated had already germinated by day 1. The difference in

percentages, show how it took longer for the seeds in the experimental groups to germinate,

validating the idea that bug repellent delays the germination process of seeds.

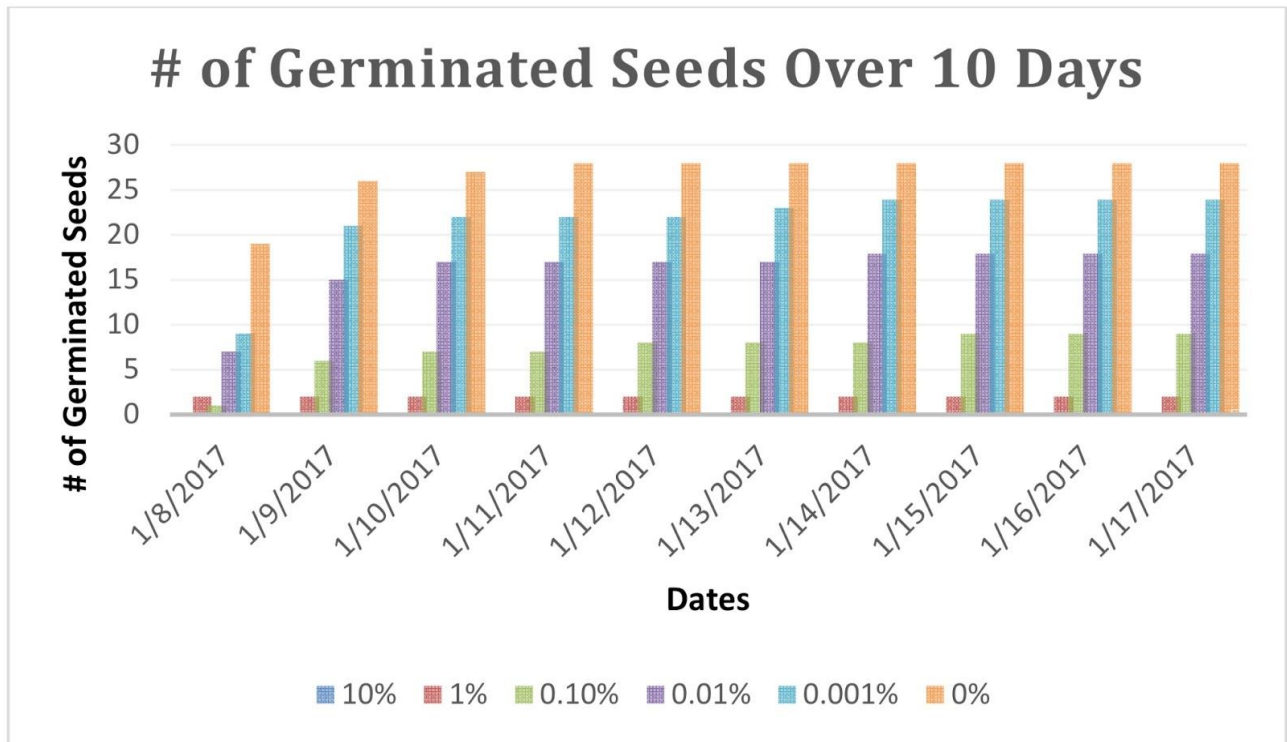
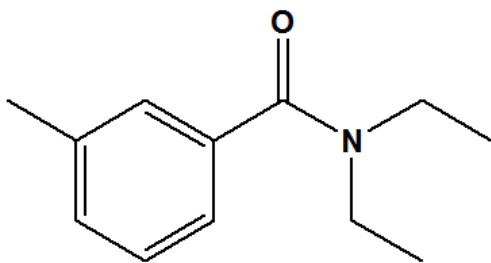


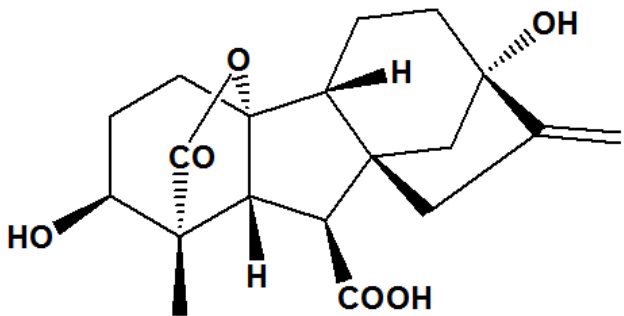
Figure 2: Germination of groups over 10 days

**The Chemistry of DEET and the Probable Inhibition Mechanism of Seed Germination**

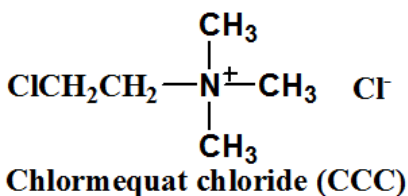
The chemical structures of DEET and related compounds are as shown below;



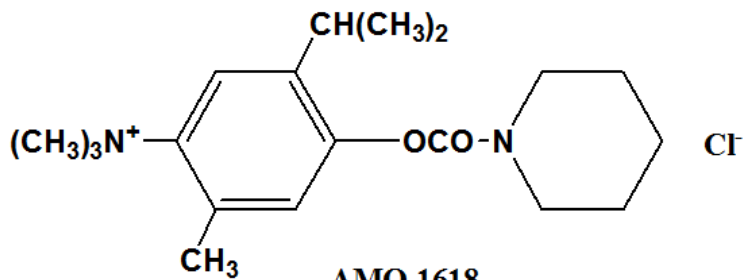
*N,N*-Diethyl-3-methylbenzamide or *N,N*-Diethyl-meta-toluamide (DEET)



Gibberillin A1



Chlormequat chloride (CCC)



AMO 1618

DEET is known to work by blocking insect olfactory receptors for 1-octen-3-ol, a volatile substance that is contained in human sweat and breath. However, more recent evidence shows that DEET serves as a true repellent in that mosquitoes intensely dislike the smell of the chemical (Syed, Z. and Leal W. S. 2008). A type of olfactory receptor neuron in special antennal sensilla of mosquitoes



that is activated by DEET, as well as other known insect repellents such as eucalyptol, linalool, and thujone, has been identified. Moreover, in a behavioral test, DEET had a strong repellent activity in the absence of body odor attractants such as 1-octen-3-ol, lactic acid, or carbon dioxide (Fox, M., and David, W. 2008).

On the other hand Gibberellins (GA) are important plant growth hormones responsible for seed germination, affecting enzyme production that mobilizes food production used for growth of new cells. This is done by modulating chromosomal transcription. In grain (rice, wheat, corn, etc.) seeds, a layer of cells called the aleurone layer wraps around the endosperm tissue (Grenman, A. K 2006). Absorption of water by the seed causes production of GA. The GA is transported to the aleurone layer, which responds by producing enzymes that break down stored food reserves within the endosperm, which are utilized by the growing seedling. GAs produce bolting of rosette-forming plants, increasing internodal length. They promote flowering, cellular division, and in seeds growth after germination. Gibberellins also reverse the inhibition of shoot growth and dormancy induced by Abscisic Acid (ABA) (Tsai, F. Y. *et al* 1997). In order to release the seed from this type of dormancy and initiate seed germination, an alteration in hormone biosynthesis and degradation toward a low ABA/GA ratio, along with a decrease in ABA sensitivity and an increase in GA sensitivity, must occur (Gerhard Leubner 2017).

Amo-1618 and Chloromequat chloride (CCC) are among the known compounds that play a great role in interfering with the normal functions of Gibberellin (GA) and Abscisic acid (ABA) which are important in seed germination. CCC and Amo-1618 interact additively in evoking retardation of hypocotyl elongation, and inhibition by CCC can be easily overcome by applying GA to the germinating seeds. The two growth retardants are known to inhibit GA biosynthesis in seeds. The available evidence suggests that CCC and Amo-1618 act at

different sites in the pathway of gibberellin biosynthesis (Baldev, B. *et al* (1965) and Dennis, D. T. *et al* (1965)). Yet, as in the case of Amo-1618, CCC-induced inhibition of growth of excised pea stem sections could be easily reversed by the application of GA (Ninnemann, H, 1964, Zeevaart J. A. D. 1964, 1966).

Based on the above information, it seems reasonable to propose that there is a greater possibility for DEET to act as a growth retardant that hinders seed germination in a similar mechanism to Amo-1618 and CCC. There is structural similarities between CCC and Amo-1618 on one hand and DEET on the other hand. DEET and Amo-1618 both contain amide functional groups in their chemical structures. In addition DEET, CCC and Amo-1618 are all nitrogen containing compounds. Based on the experimental results obtained in this study and the chemical structural resemblance between DEET and Amo-1618 and CCC it can be deduced with great confidence that DEET fall in the category of growth retardants that hinders seed germination similar to Amo-1618 and CCC.

### Conclusion

In this study the extent to which bug repellent affected the radish seeds was highly unexpected and underestimated. In experiment set 1 not even a single seed germinated amongst any of the experimental groups ranging from 25% to 100% bug repellent. This shows that higher concentrations of bug repellent has high inhibition capacity for germination of radish seeds. Experiment set 2 proved that even miniscule concentrations of bug repellent still drastically affected the germination of the seeds. With 0.01% bug repellent the amount of seeds that grew leaves decreased by more than 29% when compared to the control group. Even 0.1% repellent concentration prevented any seed from maturing to the last stage of germination and grew leaves. All experimental groups containing bug repellent as low as 0.01% concentration resulted in a statistically significant differences in the number of germinated seeds when compared with the control groups.

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