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Biotoxicity analysis of different doses of *Beauveria bassiana* (Balsamo) Vuillemin against Nymph of *Odontotermes obesus* (R.)

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ABSTRACT

Termites are soil-dwellers and carry out various activities in hidden-quarters without being detected. They pose a serious threat to agricultural, horticultural crops, forestry trees, and wooden structures. Use of entomopathogenic fungus can be a ecofriendly approach as comparable to chemical insecticides for control of various castes of termites. Entomopathogenic fungi are important natural enemies of arthropods and can be used as biological control agents. Bio efficacy of different concentrations of entomopathogenic fungus *Beauveria bassiana* (Balsamo) Vuillemin were evaluated against nymphs of termite *Odontotermes obesus* (R.) in laboratory conditions. The treatment results clearly revealed that the rate of mortality was increased with concentration and exposure period of treated dose of *Beauveria bassiana* on nymph of termite.

Key words : *Biotoxicity, Beauveria bassiana, Nymphs, Odontotermes obesus*

Introduction

Termites are well organized social insects present in terrestrial environments that feed on cellulose. Several termite species play a great ecological role in contributing appreciably to most of the ecosystems (Roonwal, 1978a). Their actions result into recycle of macronutrients such as Carbon and Nitrogen and tunneling habits helps in soil aeration, porosity (Singha *et al.,* 2010). Out of 300 species of termites known so far from India, about 35 have been reported damaging agricultural crops and buildings. Termites have been reported worldwide as one of the most important group of insects that cause significant and serious damages to crops, structures and buildings (Lee and Chung, 2003). Termites cause extensive damage to agricultural and horticultural crops, agroforestry, stored timbers, books and records, woodworks in buildings and stored products containing cellulose (Rashmi and Sundararaj, 2013). Worldwide, the anticipated loss due to termite damage is about 50 billion US$ yearly (Subekti *et al.*, 2015), although estimates vary considerably by the cropping systems followed in different environmental regions. The losses caused in India alone run into several hundred million rupees per year, and the world loss must be more than $10,000 Million.

Entomopathogenic fungi are important natural enemies of arthropods and can be used for biological control. More attention has been given to fungal pathogens of insects, which are widespread in nature and are being exploited to control pests through introduction and augmentation (Goettel *et*

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al., 2005 and Gokçe & Er, 2005). Entomopathogenic fungi differ from other insect pathogens because they are able to infect through the host's integument, so ingestion is unnecessary and infection is not limited to chewing insects (Fuxa, 1987). Termites could be effectively controlled by using pathogenic microbes. Their habitats, which are humid and of a relatively constant temperature, are ideal for microbial growth, while crowded conditions and social interaction in termite nests encourages transmission of the fungus (Delate *et al.,* 1995).

Anand *et al.* (2009) has reported that the fungal pathogen *Beauveria bassiana* infects pupae of *Spodoptera litura* in a dose–dependent manner for each of the formulations investigated which agree with the findings of the present investigation. They also reported that *Beauveria bassiana* was more infective and resulted in maximum average percent mortality among the three species viz. *Metarhizium anisopliae*, *Beauveria bassiana* and *Lecanicillium* sp. under investigation. The life cycle of a termite begins with an egg, it goes through incomplete metamorphosis, with egg, nymph and adult stages. Nymphs go through a series of moult as they grow. Nymphs first moult into workers, and then some workers go through further moulting and become soldiers or alates (Neoh and Lee, 2011). If we control nymph we can also control termite colony.

In the present investigation, bioefficacy of different concentrations of entomopathogenic fungus *Beauveria bassiana* (Balsamo) Vuillemin were evaluated against nymphs of termite *Odontotermes obesus* (R.) in laboratory conditions.

Materials and Methods

Termite collection

Underground nests of *Odontotermes obesus* (R.) located at crop fields were opened up, termites consisting of workers, soldiers and nymphs together with nest material were collected in a tray and brought to the laboratory. The termites along with the nesting mound soil were put in plastic containers and were observed in the laboratory for 1-2 months. The rearing container was covered with lids. Water was added to the containers every 3-7 days to maintain moisture. These termites were kept in petri dishes containing wet filter paper for 24 h before inoculation.

Culture of *Beauveria bassiana* (Balsamo) Vuillemin

Beauveria bassiana (Balsamo) was collected from Maharana Pratap University of Agriculture and Technology, Udaipur Rajasthan and subcultured on Sabouraud Dextrose Agar (SDA) media, in the laboratory. A spore suspension was prepared by harvesting conidia from the SDA plates just before application. Conidial count was done using Neubauer Improved Haemocytometer under a microscope for conidia/mL of dilution and recorded separately.

Inoculation

Thirty nymphs were placed on different petri plates that contained 1 mL conidial suspension of *B. bassiana* at different concentration of 4.5×10^8, 4.5×10^7, 4.5×10^6 and 4.5×10^5 conidia/mL on Whatman's filter paper disc (9 cm × 1 mm) and 1 mL of control suspension solution which contained sterile water. The nymphs were exposed to conidial suspension for 24 hours. The treated nymphs were then transferred in sterile glass Petri dishes (10 cm in diameter), lined with sterile moistened Whatman filter paper. A wood piece was placed in the petri plate for food. Both experimental and control petri dishes were kept at 28°C ± 2°C and relative humidity 75± 5 percent in BOD in the dark.

Results

Bioefficacy of different concentrations of entomopathogenic fungus *Beauveria bassiana* (Balsamo) Vuillemin were evaluated against nymphs of termite *Odontotermes obesus* (R.) in laboratory conditions. The treatment results revealed that highest concentration (4.5×10^8 conidia/mL) was significantly superior over the rest of the treatments (29.92 percent mortality) at 24 hours of treatment time (Table 1). 4.5×10^6 and 4.5×10^5 conidia/mL were at par in terms of toxicity when analyzed statistically (CD 5 percent= 4.27).

Similar trend of effectiveness was observed at 48 and 72 hours of treatment period. Highest concentration maintained its superiority even at 96 hours after exposure by registering significantly highest (95.84 percent) mortality of nymph over rest of the treatments. Second lower concentration 4.5×10^7 conidia/mL (79.17 percent mortality) proved to be next best to lower two treatments and differed significantly from remaining treatments (CD 5%= 7.31). Cent percent mortality was observed at the

Table 1. Biotoxicity of *Beauveria bassiana* (Balsamo) Vuillemin on nymphs of *Odontotermes obesus* (R.)

Treatment	Cumulative mean percent mortality ± SE					
	24 Hrs	48 Hrs	72 Hrs	96 Hrs	120 Hrs	144 Hrs
Control	5.83±1.60	10.84±2.50	15.84±0.86	17.50±0.83	19.17±1.60	19.17±1.60
4.5×10^{8} Conidia/mL (T1)	29.92±1.29	47.50±2.85	71.67±3.47	95.84±2.10	100.00±0.00	100±0.00
4.5×10^{7} Conidia/mL (T2)	23.33±1.36	38.33±3.97	61.67±2.15	79.17±2.85	95.00±1.67	100.00±0.00
4.5×10^{6} Conidia/mL (T3)	17.50±1.60	33.33±1.36	50.83±1.59	73.33±3.60	94.17±0.84	100.00±0.00
4.5×10^{5} Conidia/mL (T4)	14.17±2.50	26.67±3.04	45.00±2.89	64.17±4.17	90.00±1.36	100.00±0.00
S.E.m ±	1.725	2.871	2.376	2.951	1.254	0.713
CD (P=0.05)	4.27	7.12	5.89	7.31	3.11	1.77
CD (P=0.01)	6.35	10.57	8.74	10.86	4.61	2.62

highest concentration (4.5×10^{8} conidia/mL) after 120 hours and for rest of the treatment after 144 hours as compared to 19.17 percent in control (Fig. 1).

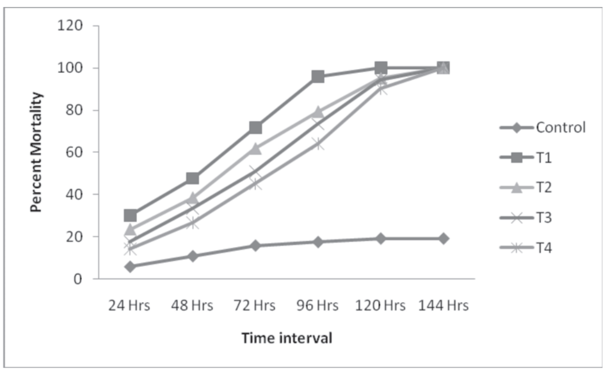

Fig. 1. Graphical representation for the biotoxicity of *Beauveria bassiana* (Balsamo) Vuillemin against nymphs of *Odontotermes obesus* (R.)

Further ANOVA also supported the results (Table 2). The mortality values were significantly increased depending on the increasing time period of treatment when the termite nymphs were exposed to different concentrations of fungus.

Probit analysis test revealed that the medium lethal conidial concentrations (LC_{50}) for nymphs of *Odontotermes obesus* (R.) were 1.25×10^{6} conidia/mL and LC_{90} was 1.19×10^{11} conidia/mL. When Chi square test for goodness of fit was conducted, the test revealed that it was non-significant ($\chi2 = 0.229$ at df =4) which means that there was no significant differences between the observed and predicted proportion of mortality. The values of lower and upper limit of LC_{50} were 2.51×10^{1} and 9.90×10^{6} conidia/mL and values of lower and upper limit LC_{90} 1.47×10^{9} and 1.90×10^{28} conidia/mL respectively. These results were also supported by graphical representation (Fig. 2).

Probit analysis was conducted to calculate LT_{50} and LT_{90} values, in hours, for each concentration of fungus. Goodness of fit tests for each concentration showed that the results were the best fit to the data of all available distributions. The Chi square values for different concentrations were 6.17, 5.04, 6.31 and 10.55 which was nonsignificant. LT_{50} and LT_{90} values for 4.5×10^{8} conidia/mL concentrations were 37.45 and 88.58 hrs. respectively, and 95 percent confi-

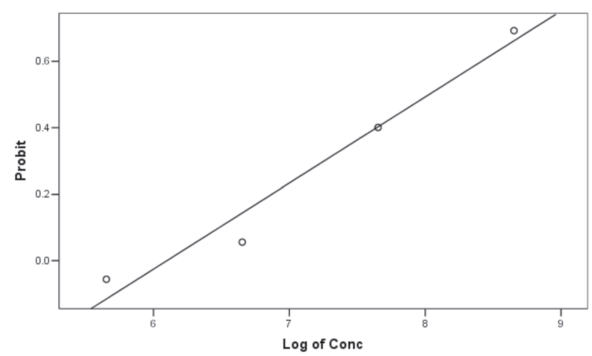

Fig. 2. Probit transformed responses for concentration v/s mortality of nymphs of *Odontotermes obesus* (R.) treated *Beauveria bassiana* (Balsamo).

Table 2. Analysis of Variance for nymphs percent mortality (Mean sum of Square)

Source of variation	df	24 Hrs	48 Hrs	72 Hrs	96 Hrs	120 Hrs	144 Hrs
Treatment	4	333.336**	756.321***	1793.423***	3472.651***	4625.608***	5227.114***
Error	15	11.90	32.961	22.591	34.827	6.294	2.033

* p< 0.05 **p<0.01, ***p< 0.001

dence limit was 13.08-55.22 and 59.46-409.13 respectively (Table 4). Amongst all selected concentrations of *Beauveria bassiana* (Balsamo) Vuillemin the 95 percent confidence intervals of LT_{50} and LT_{90} values did not overlap. The analysis also revealed that the higher the concentration, lower the values of LT_{50} and $LT_{90.}$

Discussion

Entomopathogenic fungi like *B. bassiana* normally require about 100 percent RH and about 20-30^{0}C temperature for germination, growth and sporulation (Burdeos and Villarcarlos, 1989). In the present study, the *O. obesus* inoculated with *B. bassiana* were incubated at 27-30 ^{o}C and 70-90 percent RH. These conditions apparently favored the growth of this entomopathogenous fungus thus causing high mortalities in the chosen test insect which is also the conditions in the mound built by termites. Even though there are many advantages, socialities also pose challenge with increased susceptibility to infections from aggressive microbes due to the high densities and typically low genetic diversity among colony members (Liu *et al.*, 2019).

For toxicological studies, screening of all the tested concentrations were applied against nymphs of termites and ANOVA, probit equation for $LC_{50,}$ $LC_{90,}$ LT_{50} and LT_{90} were calculated for assessment of effectiveness of the entomophathogens *Beauveria bassiana* (Balsamo) against the pest nymph of Termite.

Although percent mean mortality was quite significant for the pest suppression when different concentration of entomophathogens *Beauveria bassiana* (Balsamo) were considered. Even then, the point that has to be highlighted is that the percent mortality was directly related with the concentration of the formulated doses of bio-pesticides. The mortality increased significantly when the concentration and time period was increased.

From the biotoxicity screening experiments and statistical analysis (ANOVA), the toxicity of the *Beauveria bassiana* (Balsamo) was both time as well as concentration specific. For instance, at 4.5 × 10^5 conidia/mL, mortality of the treated castes was sig-

Table 3. Fiducial limits (95 percent), LC_{50}, LC_{90}, regression equation and chi square (χ^2) values for nymphs of *Odontotermes obesus* (R.)

Time	LC_{50} Conidia/mL	95 percent Confidence Limit		LC_{90} Conidia/mL	95 percent Confidence Limit	
		Lower Conidia/mL	Upper Conidia/mL		Lower Conidia/mL	Upper Conidia/mL
72 hrs.	1.25 × 10^6	2.51 × 10^1	9.90 × 10^6	1.19 × 10^{11}	1.47 × 10^9	1.90 × 10^{28}

Chi Sqr(χ2)[b]	Df	Result	Probit Equation
0.229	4	NS	p = -1.569 + .257 (Conc.)

[b] Pearson chi-square goodness-of-fit test on the Probit model (α= 0.05).

Table 4. Fiducial limits (95 percent), LT_{50}, LT_{90}, Probit equation and Chi square (χ^2) values for nymph's mortality. Time v/s Mortality for nymphs of termite

Concentration Conidia/mL	Chi Sqr (χ2)[b]	Probit Equation	Lethal Time (hrs.)		95 percent Confidence Limit (hrs.) Lower	Upper
4.5 × 10^8	6.17[NS]	p = -5.394 + 3.428 (Time)	LT_{50}	37.45	13.08	55.22
			LT_{90}	88.58	59.46	409.13
4.5 × 10^7	5.04[NS]	p = -5.375 + 3.238 (Time)	LT_{50}	45.71	36.99	53.73
			LT_{90}	113.72	93.74	152.28
4.5 × 10^6	6.31[NS]	p = -5.223 + 3.005 (Time)	LT_{50}	54.74	44.89	64.00
			LT_{90}	146.15	119.90	196.55
4.5 × 10^5	10.55[NS]	p = -5.851 + 3.253 (Time)	LT_{50}	62.91	46.01	78.71
			LT_{90}	155.85	119.53	251.94

[b] Pearson chi-square goodness-of-fit test on the Probit model (α= 0.05).

nificantly greater than that of the uninoculated control but at the other higher concentration the percent mortality was also higher. For nymph, mortality increased from 45.00 percent to 71.67 percent from lowest (4.5 x 10^5 conidia /ml) to highest applied concentration (4.5 x 10^8 conidia /ml) after 72 hours. Similar trend was observed for other time intervals and extent of increase in mean percent mortality.

After nymphs were infested with fungal spores, the mortality rate was less during the first two days and increased rapidly on the subsequent days. Percent mortality of termites depends on concentration of conidia. Termites that have been treated with conidia of *Beauveria bassiana* initiate to die within two days after inoculation. After six to seven days white mycelium had developed and green conidia were appeared around the insect cadavers. The reason for this is that fungal spores required the amount of time to thrive and sprout their mycelia into the termites (Krutmuanga and Mekchay, 2005) that mortality increased after the second day of exposure because specific toxin, Destruxin, from the extracts penetrated swiftly into the termites' hemocoel.

Various workers have also observed a concentration dose dependent increase in mortality with the bio-pesticides entomopathogenic fungus. Virulence is a function of fungal species. Spore concentration was found to be an important factor in the expression of virulence of the fungal conidial concentration of *B. bassiana* (Tamuli and Gurusubramanium, 2011).

Balachander *et al.* (2009) had reported that the average survival time (AST) for the termites treated with the most virulent isolate of *Metarhizium anisopliae* (Ma2) varied from 4.2 to 5.7 days across the four spore loads, while AST with the standard isolate ranged from 5.3 to 6.3 days. Two of the isolates, Ma2 and Ma13, were found to be signicantly more pathogenic to *Odontotermes* species workers than all the other species, including the standard. Habibpour *et al.* (2011) applied the combinations of *Metarhizium anisopliae* (Metschnikoff) Sorokin treated sawdust and untreated sawdust, LC_{50} and LC_{90} were 8.4×10^6 and 3.9×10^7 (spore/mL), respectively. With the use of improved bait formula and more virulent strains, achieve better control of termite colonies and enable pathogens to become a useful element in the Integrated Pest Management system.

Ansari *et al.* (2004) also reported that mortality of insect pests due to entomopathogenic fungi depends on the concentration of conidial suspension, time of exposure and temperature. Entomopathogenic fungi *M. anisopliae* isolates had low LC_{50} values (3.21×10^5 to 3.82×10^5) compared to *B. bassiana* isolates (4.39×10^5 to 5.08×10^5) which is in line with the findings of Singha *et al.* (2011)

Sahayaraj and Borgio (2010) observed 92.30 percent mortality of the *Dysdercus cingulatus* treated with green muscardine fungus, *Metarhizium anisopliae*. Researchers used *M. anisopliae* for the control of *Dysdercus cingulatus* and found 75 percent mortality after 96 hrs of exposure. The LC_{50} value of 1.25×10^6 spore/mL of the present investigation was in accordance with the study. Similarly, the entomopathogen is highly virulent against the caterpillar of *S. litura* (Gayathri *et al.,* 2010 and Joseph *et al.,* 2010).

Nagaraju *et al.* (2013) analysed and reported that the termite mortality was initially low but gradually increased and maximum mortality was observed at 36-48 hours of exposure period. *B. bassiana* and *M. anisopliae* showed 100 percent of mortality of treated termites, compared to *Penicillium notatum* and *Aspergillus flavus* lowest LT_{50} values. In *Odontotermes obesus* initially mortality was low but as the time advanced gradually the mortality of termites were also increased and it was peak at 36-48 hours of exposure and low mortality at 60 hours.

Conclusion

The mortality rate of nymph increased significantly when the concentration and time period was increased.

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References

Anand, R., Prasad, B. and Tiwary, B. N. 2009. Relative susceptibility of *Helicoverpa armigera* pupae to selected entomopathogenic fungi. *Biological Control*. 54: 85-92.

Ansari, M.A., Vestergaard, S., Tirry, L. and Moensa, M. 2004. Selection of a highly virulent fungal isolates, *Metarhizium anisopliae* CLO 53, for controlling *Hoplia philanthus*. *J. Invertebrate Pathol.* 85: 89-96.

Balachander, M., Remadevi, O.K., Sasidharan, T.O. and Sapna Bai, N. 2009. Infectivity of *Metarhizium anisopliae* (Deuteromycotina: Hyphomycetes) isolates to the arboreal termite *Odontotermes* sp. (Isoptera: Termitidae*). International Journal of Tropical Insect Science*. 29(4) : 202–207.

Burdeos, A.T. and Villacarlos, L.T. 1989. Comparative pathogenicity of *Metarhizium anisopliae*, *Beauveria bassiana* and *Paecilomyces lilacinus* to adult sweet potato Weevil, *Cylas formicarius* (F.) (Curculionifae). *The Philippines Entomologist*. 7 : 561-571.

Delate K. M., Grace J. K. and Tome C. H. M. 1995. Potential use of pathogenic fungi in baits to control the Formosan subterranean termite (Isopt., Rhinitermitidae). *Journal of Applied Entomology*. 119: 429–433.

Fuxa, J.R. 1987. Ecological considerations for the use of Entomopathogens in IPM. *Annual Review of Entomology.* 32: 225-51.

Gayathri, G., Balasubramanian, C., Vinayaga Moorthi, P. and Kubendran, T. 2010. Larvicidal potential of *Beauveria bassiana* (Balsamo) Vuillemin and *Paecilomyces fumosoroseus* (Wize,) Brown and Smith on *Culex quinquefasciatus* (Say). *Journal of Biopesticides*. 3(1) : 147-151.

Goettel, M.S., Eilenberg, J. and Glare, T. 2005. Entomopathogenic fungi and their role in regulation of insect populations. In: *Comprehensive Molecular Insect Science* (Eds. L.I., Gilbert, K., Iatrou & S.S., Gill), Amsterdam. 361–405.

Gokçe, A. and Er. M.K. 2005. Pathogenicity of Paecilomyces spp. to the glasshouse whitefly, *Trialeurodes vaporariorum,* with some observations on the fungal infection process. *Turkish Journal of Agriculture and Forestry.* 29 : 331-339.

Habibpour, B., Cheraghi, A. and Mossadegh, M.S. 2011. Evaluation of cellulose substrates treated with *Metarhizium anisopliae* (Metschnikoff) Sorokin as a biological control agent against the termite *Microcerotermes diversus* Silvestri (Isoptera: Termitidae). *J. Ent. Acarol. Res.* Ser. II, 43 (2) : 269-275.

Joseph, I., Edwin Chellaiah, D. and Ranjith Singh, A.J.A. 2010. Studies on the influence of *Beaueria bassiana* on survival and gut - flora of groundnut caterpillar *Spodoptera litura* Fab. *Journal of Biopesticides*,3(3): 553–555.

Krutmuanga, P. and Mekchay, S. 2005. Pathogenicity of entomopathogenic fungi *Metarhizium anisopliae* against termites. *Proceedings of the Conference on International Agricultural Research for Development, October 11-13, 2005,* Stuttgart-Hohenheim, Germany.

Lee, C. Y. and Chung, K. M. 2003. Termites. In: *Urban Pest Control - A Malaysian Perspective.* Second Edition (Lee, C. Y., J. Zairi, H. H. Yap and Chong, N. L. eds.). 99-111.

Liu, L., Zhao, X.Y., Tang, Q.B., Lei, C.L. and Huang, Q.Y. 2019. The mechanisms of social immunity against fungal infections in eusocial insects. *Toxins.* 11 : 244.

Nagaraju, K., Sundararaj, R. and Meenakshi, B.C. 2013. *In vitro* Studies of Entomopathogenic Fungus against Termites. *Research Journal of Agricultural Sciences*. 4(2): 305-307.

Neoh, K.B. and Lee, C.Y. 2011. Developmental stages and caste composition of a mature and incipient colony of the drywood termite, *Cryptotermes dudleyi* (Isoptera: Kalotermitidae). *Journal of Economic Entomology.* 104 (2): 622–28.

Rashmi, R. S. and Sundararaj, R. 2013. Host range, pest status and distribution of wood destroying termites of india. *Journal of Tropical Asian Entomology.* 2 : 12–27.

Roonwal, M. L. 1978a. Bioecological and economical observations on termites of Peninsular India. *Zeitschrift für Angewandte Entomologie*. 85 : 15–30.

Sahayaraj, K and Borgio, J. F. 2010. Virulence of entomopathogenic fungus *Metarhizium anisopliae* (Metsch.) Sorokin on seven insect pests. *Indian Journal of Agricultural Research*. 44(3) : 195–200.

Singha, D., Singha, B. and Dutta, B.K. 2010. Potential of *Metarhizium anisopliae* and *Beauveria bassiana* in the control of tea termite *Microtermes obesi* Holmgren in vitro and under field conditions. *J Pest Sci.* 66–70.

Singha, D., Singha, B. and Dutta, B.K. 2011. Potential of *Metarhizium anisopliae* and *Beauveria bassiana* in the control of tea termite *Microtermes obesi* Holmgren *in vitro* and under field conditions. *J. Pest Sci.* 84 : 69-75.

Subektia, N., Yoshimurab, T., Rokhmanc, F. and Masturd, Z. 2015. Potential for subterranean termite attack against five bamboo species in correlation with chemical components. *Procedia Environmental Sciences.* 28 : 783–788.

Tamuli, Ajit, K. and Gurusubramanium, G. 2011. Entomopathogenicity of white Muscardine Fungus *Beauveria bassiana* (Balls.) Vuill. (Deuteromyotina: Hyphomycetes) (BBFF-135) Against *Odontotermes* (Rambur) (Isoptera: Termitidae). *Assam University Journal of Science & Technology.* 7(1) : 118-125.