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Bioinsecticidal activity of *Vitex negundo* L. (Family: Verbenaceae) leaf extracts against *Sitophilus granarius* L. in stored maize grains

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Abstract
The insecticidal property of dry leaves of *Vitex negundo* against the *Sitophilus granarius* in the laboratory at (28±3) ^{0}C and (78±3) % r. h. with Soxhlet extracted extracts of *V. negundo* leaves in ethanol, methanol, chloroform, acetone and n-Hexane were tested and found very effective to control the store grain insect pest. The biopesticidal effects of extracts in different solvents were compared by calculating LD_{10}, LD_{50}, LD_{90} and LD_{99} values for 96 hrs. The Probit analysis of data demonstrated that LD values for ethanol, methanol, chloroform, acetone and n-Hexane extracts were LD_{10} – 1.216, 1.141, 0.9298, 1.265, 0.5398 ml/Kg; LD_{50} – 2.997, 3.106, 2.490, 3.727, 1.302 ml/Kg; LD_{90} – 7.392, 8.457, 6.667, 10.98, 3.142 ml/Kg and LD_{99} –15.43, 19.14, 14.88, 26.50, 6.439 ml/Kg respectively, for 96 hrs. These results demonstrated that the mortality increased with increase in concentration of extract and exposure time. There was nearly zero fecundity and next generation was not observed in treated grains. The extracts of *V. negundo* leaf may be of high value in grain storage against *S. granarius*, especially in subsistence agriculture where the plants are locally available to local farmers. Hence, we conclude that the ethanol, chloroform and n-Hexane extracts from *V. negundo* leaf are more effective and useful. It served as being a potential insecticidal agent and could be applicable to the management of populations of stored-product insects particularly against the infestation by granary weevil, *S. granarius* after proper dose formulation.

Keywords: *Sitophilus granarius*, *Vitex negundo*, soxhlet, bioinsecticide, stored grain, toxicity, mortality

1. Introduction
Insect pests of stored products are create the serious problem throughout the world, because they reduce the quantity and quality of stored grains. The cereals are badly infested by the most destructive primary stored grain weevils, *Sitophilus granarius*, *Sitophilus oryzae* and *Sitotroga cerealella* in warm climate areas. They multiplies very rapidly and cause up to 30-40% cereal grain loss in India as well as in other countries at conditions favorable to their development at about 25–35 ^{0}C and generally low relative humidity [1, 2]. Both the adult and larva feed voraciously on a variety of stored cereal grains viz. maize, rice, wheat, and sorghum causing serious losses, particularly in the monsoon. The species *Sitophilus zeamais* affects cereals quantitatively as well as qualitatively and contaminates grain with excrement and exuviae [3]. Few natural plant products that overcome insect damage to animals or plants by rendering them unattractive or offensive are called repellents. Their pungent odour, make them unattractive to host plants. Use of insect repellent offers a hope for protection of stored grains from insect attack. They are more effective, more persistent and more economical than the existing synthetics [4]. In the integrated protection of stored-products, phytochemicals may be used for (i) pest prevention by repelling pests from goods, (ii) early pest detection, attracting pests to lures or (iii) pest control by using toxic compounds [5].
The Nirgundi, *Vitex negundo* is an aromatic well-known large shrub or small tree mainly found on bank of rivers in villages and almost found throughout the India. The phytochemical screening of *Vitex negundo* revealed the presence of alkaloids, steroids, flavonoids, amino acids, phenols, quiones and starch [6]. Research aiming towards the development of new agents for insect-pest control based on natural products is increasingly important because it has consequences in integrated as well as biological pest management in agriculture [7]. Plant secondary metabolites play an important role in interactions of plant–insect and therefore such

compounds may have hormonal, insecticidal or antifeedant activity against insects [8]. The phytochemical analysis of *V. negundo* revealed that presence of various active phytochemicals. [9] Bameta *et al.*, (2019), reported that chloroform crude extract of *V. negundo* leaves contain maximum number of phytoconstituents viz. reducing sugar, saponins, steroid and coumarin followed by hexane i.e. reducing sugar, phenol and coumarin. While ethanol, methanol and petroleum ether shows the presence of phenol, saponin, coumarin, respectively.

There is need for safe but effective, biodegradable pesticides with non-toxic effect on non-target organisms. Botanical insecticides are broad-spectrum in pest control and many are safe to apply.

2. Materials and Methods

2.1. Test insects

The rice weevil, *Sitophilus granarius* tested in this study was obtained and laboratory culture was maintained on pest free whole maize grains. Adult 1±2 week old weevils (male and females) were separated and kept in the experimental environment to acclimatize for 5±7 days before commencement of the bioassays.

2.2. Test materials

Leaves of *V. negundo* plant not exposed to any pesticide were collected from local, rural region of Aurangabad (MS) district. After harvesting, test materials were air dried in room conditions.

2.3. Preparation of test materials for bioassay

The bioassay was carried out by applying the test materials on maize grains. For this purpose, test material was ground separately in a motorized high speed grinder and sieved through a 72 mm aperture mesh to obtain fine powder. Then a known amount of test material was extracted by Soxhlet's extractor i.e. 1:10 ratio in the solvents ethanol, methanol, chloroform, acetone and n-Hexane, separately.

2.4. Toxicity of *V. negundo* leaf extract to adult insects

The stock culture of *S. granarius* reared in laboratory condition was used for experimentation. 50 gms of the pest free whole maize grains were taken in each of the plastic containers. The dried maize grains were held in a controlled environment room at 28±3 ^{0}C and 78±3% r. h. for 5±7 days to equilibrate them to 14% moisture content. The leaf extract of *V. negundo* was dissolved thoroughly with respective solvents to make 10 ml volume. From this stock, test concentrations 0.2, 0.4, 0.6 and 0.8 ml extract/50g were added in each plastic container containing 50 gm of whole maize grains. The untreated maize grains were kept as a control. The plastic containers were tied with muslin cloth and kept open for 48 hours in well ventilation room to evaporate the solvent. After 48 hours, 5 pairs (1±2 week old) of *S. granarius* adults acclimatized from stock culture were released in each of the control and experimental containers. The containers were completely covered with a piece of moist muslin cloth and fixed with the help of rubber band to prevent escapes. The whole experiment was repeated thrice.

2.5. Statistical analysis

The percentage mortality was calculated and corrected according to Abbott, (1925) [10] after every 24 hrs. and data collected was analyzed. The data transformed into Finney's Probit values for the determination of LD_{10}, LD_{50}, LD_{90} and LD_{99} values for 96 hrs [11].

3. Observation and Results

The *V. negundo* leaf extract has strong toxicity against *S. granarius* adults. The mortality increased with raising concentration from 4 to 16 ml extract/Kg and with exposure times of 24 and 96 h. The range of statistical calculations and determination of LD_{10}, LD_{50}, LD_{90} and LD_{99} values as per [11] Finney's, (1971) are given in Table No.1. The LD_{10}, LD_{50}, LD_{90} and LD_{99} values after calculation for ethanol extract were 1.216, 2.997, 7.392, 15.43 ml/Kg respectively. The LD_{10}, LD_{50}, LD_{90} and LD_{99} values for methanol extracts were 1.141, 3.106, 8.457 and 19.14 ml/Kg respectively. For chloroform extract the respective LD values were 0.9298, 2.490, 6.667 and 14.88 ml/Kg, for acetone extract, 1.265, 3.727, 10.98 and 26.50 ml/Kg. For n-Hexane extract the values were 0.5398, 1.302, 3.142 and 6.439 ml/Kg respectively. If we compare all values, the n-Hexane extracts of *V. negundo* acts as a potent insecticide against *S. granarius* and can be used for developing baits or to apply directly for managing this pest during storage. The acetone extracts of *V. negundo* shows higher lethal dose values; therefore it is not suitable even though it has biopesticidal activity against *S. granarius*.

The graph regarding empirical/improved expected probit against the log of concentration are given in (Figure 1-5) as regression and provisional lines for LD_{10}, LD_{50}, LD_{90} and LD_{99} values of *Sitophilus granarius* after the exposure to ethanol, methanol, chloroform, acetone and n-Hexane extract of *Vitex negundo* leaves for 96 hours.

3.1 Effects on fecundity and next generation (F_1)

The growth, fecundity, life cycle of insect depends on temperature and relative humidity favorable to them. Out of infected grains till death of adult *S. granarius*, very less or none of them shows adult insects emergence in all the solvent extracts cases. The rate of infected maize grains and adult emergence decreased with raising concentration from 4 to 16 ml extract/Kg and with exposure times of 24 and 96 hrs. In next generation, after exposure to all extracts, the emergence of adults *S. granarius* was observed in control and not in treated maize grains, because it may due to the bioactive compounds present in extracts and insects response to these secondary metabolites. Table No. 2 to 6 shows the effects of different solvent extracts of *Vitex negundo* leaves on the reproductive activity of the stored grain pest, *Sitophilus granarius.*

4. Discussion

Plant powders, their extracts and isolated pure compounds are effective and considered ecofriendly in storage insect's control. The bioactivity of plant extracts against storage insects of maize have been studied by numerous authors [12-14]. The present results indicate that the ethanol, methanol, chloroform, acetone and n-Hexane extracts of *V. negundo* leaves were effective against *S. granarius*. [15] Jawalkar *et al.*, (2016), reported that the LD_{10}, LD_{50} and LD_{90} values calculated for ethanol extract of *D. stramonium* seeds were 2.962 ml/Kg, 8.594 ml/Kg and 27.94 ml/Kg respectively. Similarly, the LD_{10}, LD_{50} and LD_{90} values calculated for chloroform extracts of *D. stramonium* seed were 3.080 ml/Kg, 7.379 ml/Kg and 17.67 ml/Kg respectively. For acetone extracts of *D. stramonium* seed it was 0.4752 ml/Kg, 1.185 ml/Kg and 2.957 ml/Kg respectively. [16] Sahaf *et al.*, (2008),

reported that the fumigant toxicity of *V. pseudo-negundo* oil was toxic to *S. oryzae* and *T. castaneum*. The highest concentration (925.9 µL/L air) of the oil was able to induce more than 50% mortality after 6 h and achieved a level of 100% at 12 h after treatment. [17] Lee *et al.*, (2001), also reported the potent fumigant toxicities of two essential oils i.e. eucalyptus and rosemary against the rice weevil. The oils lavender, thyme, ylang-ylang and grape fruit showed weak fumigant toxicity. [18] Derbalah *et al.*, (2012), reported that the extracts from the plant *Caesalpinia gilliesii* (100%) was the most effective against the pests *Sitophilus oryzae* adults followed by *Chrysanthemum frutescens* (95.6%), *T. populnea var. acutiloba* (88%), *Euonymus japonicas* (85%), *Bauhinia purpurea* (75%), *Cassia senna* (80%) and *Cassia fistula* (70%) respectively. [19] Rajashekar *et al.*, (2010), reported that the methanolic root extract of *Decalepis hamiltonni* was effective against the *Rhyzopertha dominica, Sitophilus oryzae, Stegobium paniceum, Tribolium castaneum* and *Callosobruchus chinensis.* [20] Khani *et al.*, (2011), reported that the petroleum ether and chloroform extracts of *P. nigrum* and petroleum ether extracts of *J. curcas* caused highest mortality rates for *Sitophilus oryzae* based on LC_{50} values after 72 h. Petroleum ether and chloroform extracts of *P. nigrum* were more toxic with 99.56, 93.56% mortality rates than petroleum ether extract of *J. curcas* with 66.00% mortality rate, respectively.

According to [21] Saljoqi *et al.*, (2006) the effect of ethanolic extracts of Bakain drupes (*Melia azedarach*), Leaves of Habulas (*M. communis*), Leaves of mint (*Mentha longifolia*), Bakain leaves, Harmal shoots and seeds (*P. harmala*) and Roots of lemon grass (*C. citratus*) had repellent and lethal effects against rice weevil, *Sitophilus oryzae.* [22] Ileke and Ogungbite (2014), reported that powders and extracts of *Azadirachta indica, Zanthoxylum zanthoxyloides, Anacardium occidentale* and *Moringa oleifera* has high mortality effect against *Sitophilus oryzae, Oryzaephilus mercator* and *Rhyzopertha dominica*. All the extracts, at all tested concentrations, achieved 100% mortality of *S. oryzae* within 72 hrs. of exposure except extract of *M. oleifera* at 2 and 4% which achieved 72% and 87.59% mortality, respectively. [23] Ziaee *et al.*, (2014), demonstrated fumigant toxicity of *C. cyminium* oil loaded nano gels (OLNs) against *S. granarius* and *T. castaneum* and revealed OLNs were most toxic and encapsulation improved the persistence of the oil. [24] Li *et al.*, (2000), reported that isolated fraction from leaf of *Eupatorium adenophorum* showed insecticidal activity against *S. oryzae* (LD_{50} = 15.5 mg/L). [25] Jayasekara *et al.*, (2005) reported that root powder of *Securidaca longepedunculata*, its methanol extract and isolated compound methyl salycilate has repellent and toxic properties against *S. zeamais* adults. According to [26] Potenza *et al.*, (2006), acetone extracts of *Dahlia pinnata*, *Ruta graveolens* and *Dieffenbachia brasiliensis* at 5% concentration gave 87.0, 80.0 and 75.0% mortality for *S. zeamais* adults, respectively when tested for contact insecticidal activity. [27] Huang *et al.*, (2007), reported 100% mortality of *R. dominica* at 48 h post-treatment with LD_{50} - 19.94 µg/cm^2 after 72 h treatment when treated with ethanol extracts of *Trigonella foenum-graecum* extract at 0.39 mg/cm^2. [28] Boeke *et al.* (2004) reported that plant extracts inhibit the development of eggs and immature stages present inside the seed. [29] Dwivedi and Garg (2000), reported the acetone extract of *Ipomoea palmata* showed 57.8% egg mortality of *C. cephalonica* at 100.0% concentration. In other work, flower extracts of *L. camara* exhibited higher larval mortality, reduced fecundity and prolonged development period of *R. dominica* [30]. [31]Khani *et al.*, (2012a), reported that petroleum ether extract of *P. nigrum* and *Jatropha curcas* at concentrations of 2~10 µl/g showed strong inhibition on egg hatchability and adult emergence of *C. cephalonica*. [32] Zambare *et al.*, (2012) reported that chloroform extract of *Argemone mexicana* at 4 ml concentration inhibited 60.02% egg hatching of *C. cephalonica*. [33] Fouad *et al.*, (2014) reported that extracts of *Tithonia diversifolia* at 1% w/w discouraged egg laying and larval mortality of *S. cerealella*; potentiality of this plant extract is due to the presence of sesquiterpenes, lactones [34]. In another work, [35] Ileke (2014), reported that powders and extracts of *Capsicum frutescens*, *Cymbopogon citratus*, *Moringa oleifera* and *Anacardium occidentale* at the rate of 1, 2 and 3% concentration prevented egg hatching of *S. cerealella*. [36] Thein *et al.* (2013), reported that crude extracts of Citronella at 20% concentration had the strongest repellent effect on *Sitophilus* spp. and plant extract from *Alpinia pyramidata* at 7.5% provided protection to maize grain by completely inhibiting the F1 progeny emergence. Significant insecticidal activity against *T. castaneum* larvae and adults was observed by [37] Jbilou *et al.*, (2006) with crude methanol extract from *P. harmala*, followed by extracts of *A. iva*, *Ari baetica* and *R. raphanistrum*. They reported the extract of *R. raphanistrum* reduced significantly the progeny production F1, while extracts of *P. harmala*, *A. iva* and *Ari baetica* inhibited completely F_1 progeny production.

Table 1: The LD_{10}, LD_{50}, LD_{90} and LD_{99} values and regression equation of *Vitex negundo* leaf extract to *Sitophilus granarius.*

Name of plant	Solvent	Time of exposure in hrs.	Regression equation $Y = \overline{(y)} + b\,(x - \overline{x})$	LD_{10} values in ml/Kg	LD_{50} values in ml/Kg	LD_{90} values in ml/Kg	LD_{99} values in ml/Kg
Leaf extract of *Vitex negundo*	Ethanol	96	$Y = 3.2700x - 0.1706$	1.216	2.997	7.392	15.43
	Methanol	96	$Y = 2.9461x + 0.6037$	1.141	3.106	8.457	19.14
	Chloroform	96	$Y = 2.9961x + 0.8169$	0.9298	2.490	6.667	14.88
	Acetone	96	$Y = 2.7307x + 0.709$	1.265	3.727	10.98	26.50
	n-Hexane	96	$Y = 3.3511x - 1.2645$	0.5398	1.302	3.142	6.439

Table 2: Effect of Ethanol extract of *Vitex negundo* leaves on the mortality, fecundity and development of the stored grain pest, *Sitophilus granarius.*

Dose in ml/Kg	Mortality (%) in hrs.		Infected grains till death of adults	Emergence of adults up to 42 days
	24	96		
Control	-	-	78.66±3.05	17±2.66
4	-	50	40.33±4.16	4±1.33
8	20	90	33±4.58	Nil
12	40	100	24.33±4.04	Nil
16	50	100	Nil	Nil

± indicates S. D. of three observations.

Table 3: Effect of Methanol extract of *Vitex negundo* leaves on the mortality, fecundity and development of the stored grain pest, *Sitophilus granarius*

Dose in ml/Kg	Mortality (%) in hrs.		Infected grains till death of adults	Emergence of adults up to 42 days
	24	96		
Control	-	-	78.66±3.05	17±2.66
4	20	60	43.33±6.11	5±0.87
8	30	90	49±4.58	2±0.00
12	40	100	Nil	Nil
16	60	100	Nil	Nil

± indicates S. D. of three observations.

Table 4: Effect of Acetone extract of *Vitex negundo* leaves on the mortality, fecundity and development of the stored grain pest, *Sitophilus granarius.*

Dose in ml/Kg	Mortality (%) in hrs.		Infected grains till death of adults	Emergence of adults up to 42 days
	24	96		
Control	-	-	78.66±3.05	17±2.66
4	10	60	27.33±7.02	2±0.87
8	20	80	Nil	Nil
12	40	100	Nil	Nil
16	40	100	Nil	Nil

± indicates S. D. of three observations.

Table 5: Effect of Chloroform extract of *Vitex negundo* leaves on the mortality, fecundity and development of the stored grain pest, *Sitophilus granarius.*

Dose in ml/Kg	Mortality (%) in hrs.		Infected grains till death of adults	Emergence of adults up to 42 days
	24	96		
Control	-	-	78.66±3.05	17±2.66
4	30	70	Nil	Nil
8	40	100	Nil	Nil
12	50	100	Nil	Nil
16	50	100	Nil	Nil

± indicates S. D. of three observations.

Table 6: Effect of n-Hexane extract of *Vitex negundo* leaves on the mortality, fecundity and development of the stored grain pest, *Sitophilus granarius.*

Dose in ml/Kg	Mortality (%) in hrs.		Infected grains till death of adults	Emergence of adults up to 42 days
	24	96		
Control	-	-	78.66±3.05	17±2.66
4	-	20	21±4	8±1.73
8	20	50	18.33±3.05	3±1.33
12	30	80	Nil	Nil
16	40	100	Nil	Nil

± indicates S. D. of three observations.

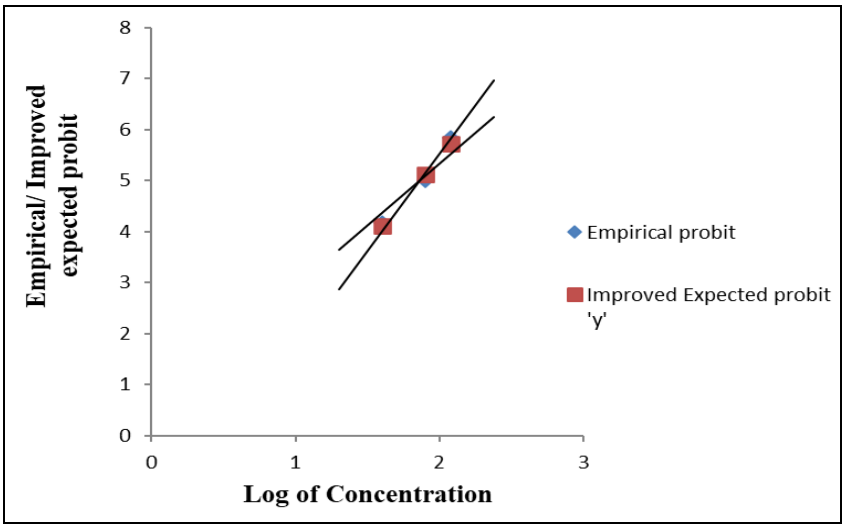

Fig 1: Regression and Provisional line for LD_{10}, LD_{50}, LD_{90} and LD_{99} values of *Sitophilus granarius* after the exposure to ethanol extract of leaf of *Vitex negundo* for 96 hours.

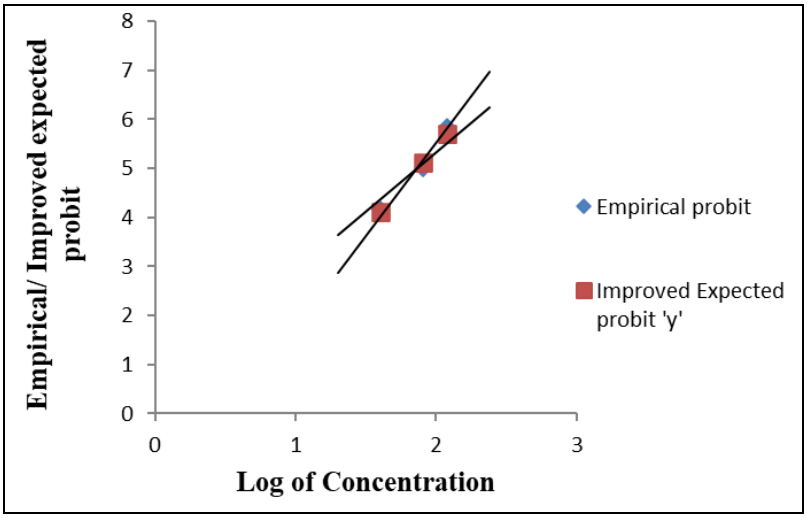

Fig 2: Regression and Provisional line for LD_{10}, LD_{50}, LD_{90} and LD_{99} values of *Sitophilus granarius* after the exposure to methanol extract of leaf of *Vitex negundo* for 96 hours.

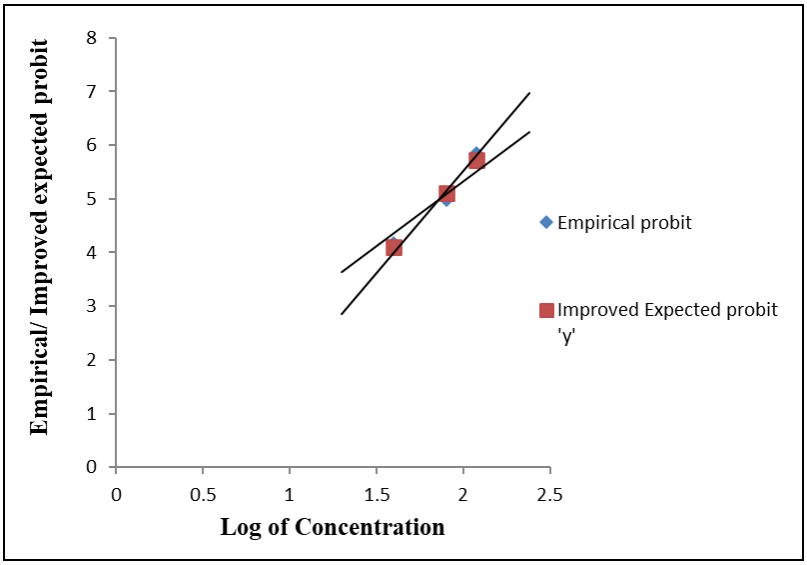

Fig 3: Regression and Provisional line for LD_{10}, LD_{50}, LD_{90} and LD_{99} values of *Sitophilus granarius* after the exposure to chloroform extract of leaf of *Vitex negundo* for 96 hours.

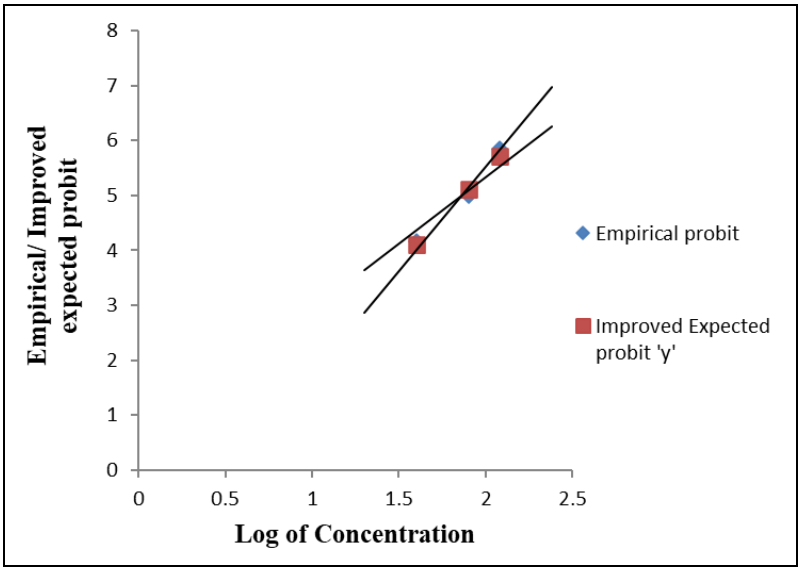

Fig 4: Regression and Provisional line for LD_{10}, LD_{50}, LD_{90} and LD_{99} values of *Sitophilus granarius* after the exposure to acetone extract of leaf of *Vitex negundo* for 96 hours.

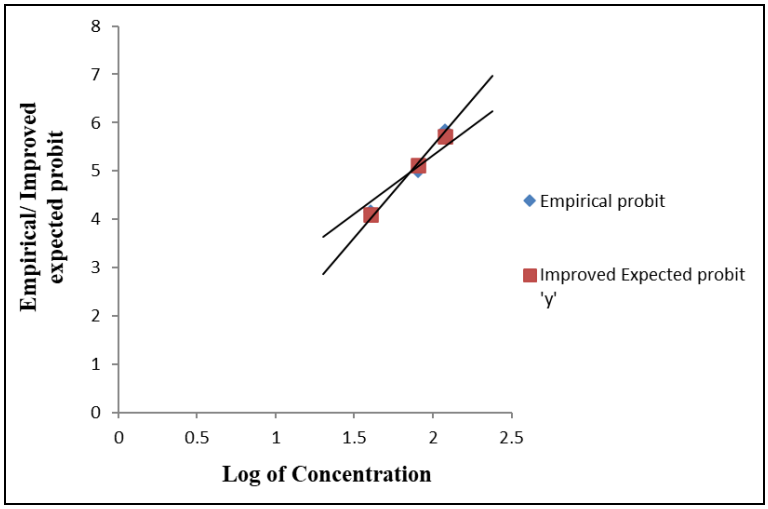

Fig 5: Regression and Provisional line for LD_{10}, LD_{50}, LD_{90} and LD_{99} values of *Sitophilus granarius* after the exposure to n-Hexane extract of leaf of *Vitex negundo* for 96 hours

5. Conclusion

The results obtained from this bioassay revealed that all extracts of *Vitex negundo* leaf have shown varying levels of insecticidal potential against *Sitophilus granarius*. The ethanol, chloroform and n-Hexane extracts from *V. negundo* leaf are more effective and useful for managing the population of granary weevil during storage. The importance of *V. negundo* for the control of storage pest such as *S. granarius* has been proved. This can be used as an alternative for the chemical insecticides and with this the safer storage of maize grains will be ensured.

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