Indian J.Sci.Res. 8(1) : 63-70 , 2017
ISSN : 0976-2876 (Print)
ISSN : 2250-0138 (Online)

BIO-PESTICIDAL EFFICACY OF PURIFIED FRACTIONS OF *Solanum nigrum* (L) WHOLE PLANT EXTRACTS ON INSECT *Tribolium castaneum* (HERBST.) - A RED FLOUR BEETLE

RITU NAMDEO[a], RAJENDRA CHAUHAN[b], M. L. GOUND[a], KISHOR SHENDE[c] AND KAPIL K. SONI[1a]

[a]Department of Biosciences, Barkatullah University, Bhopal, M.P., India
[b]Department of Zoology, Govt. Motilal Vigyan Mahavidyalaya, Bhopal ,M.P., India
[c]Department of Biotechnology and Bioinformatics Centre (Sub-DIC), Barkatullah University, Bhopal ,M.P., India

ABSTRACT

Since many centuries peoples are struggling to protect stored grains which are fed by insects. Synthetic insecticide has led many problems such as environmental disturbances, pest resurgence, pest resistance to pesticides and lethal effects on non-target organisms including man. Therefore, natural products isolated from plants have become as potential sources of new pesticides. In the present study, the insect *Tribolium castaneum* (Herbst) is a damaging pest of wheat grains and its flour. Looking to the economic importance of host plant *Triticum aestivum* as edible crop that is being fed by the pest *Tribolium castaneum,* it was thought important to develop bio-pesticides of plant origin. Plant *Solanum nigrum* (L.) after identification and authentication from RHMD, New Delhi, collected from the Arera colony area of the Bhopal and shade dried and pulverized to get powder which was extracted in different solvents through Soxhletion and percentage yield was noted down 1.67% in benzene. Obtained extract were fractionated by TLC and RF value were measured viz. 0.12, 0.40, 0.48, 0.89 in Petroleum ether :Toluene: Ethyl acetate (50:30:20) and Rf value 0.59, 0.68, 0.78, 0.90 were measured in Chloroform and methanol (50:50) and column chromatography was also sought to get purified fraction which was applied on adult insects *Tribolium castaneum* L. Results showed that purified fractions of *Solanum nigrum* benzene extract at 100% concentration was reported with 86.67±15.28% mortality that is compared with standard drug Parad Tikdi which showed 6.67±11.5% mortality and LC_{50} value was reported 51.50% and upper and lower values at 95% confidence interval are 62.44% and 40.82%, respectively

KEY WORDS : Bio-pesticides, Bio-insecticides, Phyto-chemicals, Mortality, *Solanum nigrum*, Tribolium castaneum

Storage of agricultural food grains is much more important task before coming of the monsoon season not only in houses but also in ware houses. These commodities provide perfect breeding places for a variety of pests, particularly storage beetle. The quality and quantity of these commodities attacked by the storage grain pests especially *Tribolium castaneum* resulted in the reduction of volume, germination damage in grains and sufficient weight loss (Phillips and Throne, 2010*,* Nadeem et al., 2012). The magnification of insect infestation is supported by a number of uncontrolled ecological aspects and reduced storage technologies (Upadhyay and Ahmad, 2011). A basic need of the optimal insect pest management measures are chemical control methods which are still being used even the adverse and toxic effects of synthetic insecticides are well known. Moreover, environmental pollution is a main hazard for the sustainability of the ecological biome due to the uncontrolled use of synthetic pesticides and therefore, in the present scenario plant extracts present a safer solution to all these issues (Sagheer et al., 2013).

The ancient man had deployed different methods of control, including prayers, magic spells, cultivation systems, mechanical practices, as well as application of organic and inorganic substances to protect his crops from the attack of weeds, diseases and insect pests (Kulkarni et al., 2009). Between 500BC and in the 19th century, a number of substances classified as pesticides and defined as any substance and mixture of substances intended for preventing, destroying, repelling or mitigating any pest which were used to control pests which are harmful organism that may be insects or rodents.

The necessary step at recent times is to give consideration to find unconventional substitutes for the control of storage insects pests. Utilizing the plant products and the secondary metabolites present in various plants as natural control materials is one of the methods for the management (Isman, 2000). It is reported by Bakkali et al. (2008) that secondary metabolites which defend plants from external hazard are natural defensive products. Some plants extracts have evidenced as effective against several storage insects especially *Tribolium castaneum*, that is well known

[1]Corresponding author

as the red flour beetle (Sagheer et al., 2011, Khan et al., 2013).

The plant kingdom contains at least five Lac different species and they produce a very diverse range of secondary metabolites (Farnsworth, 1990). Only about 5-10% of these have been examined till now chemically for biologically active compounds and more than 2 thousands plant species have been reported to possess insect/pest control properties (Grainge and Ahmed, 1988). Plant secondary metabolism produces a very varied range of metabolites including terpenoids, alkaloids, polyacetylenes, flavonoids, phenols, polyenes, saponins, glycosides, tannins, resins, amino acids and sugars offering a rich diversity of biologically active compounds. Many of these are believed to have evolved to protect the plants and stored grains from attack by insect pest.

MATERIALS AND METHODS

In the present study, whole plant of *Solanum nigrum* (L.), commonly known as Makoy (Fig.1) was collected from Arera colony Bhopal after identification and authenticated from RHMD, NISCAIR, New Delhi (Ref. No.2554-133-2/NISCAIR/RHMD/Consult/2014), which was shade dried at room temperature and pulverized to get powder of 40-60 mesh size and loss in weight of the materials was measured (Table 1) then extracted in benzene, methanol and distilled water solvents through Soxlhet apparatus (Sajid et al., 2012) and percentage yield was calculated (Table 2). Obtained crude extracts were separated and purified by thin layer and column chromatography (Tswett 1906, Dubey et al., 2014, Deepthi and Pradeep 2016) by applying different solvent system to get purified fractions (Ramachandran et al. 2014). The spots developed on TLC plates (Lux plates, Merck) was visualized in UV chamber at 356nm, then in Iodine chamber and in visible light and RF values of all spots were calculated (Table 3, Fig 2 (a, b, c) by the formula of Brimley and Barrett (1953) as described below:

$$Rf = \frac{\text{Distance travelled by solutes}}{\text{Distance travelled by solvents}}$$

Then, Column chromatography of the benzene extract was sought in Petroleum ether: Toluene: Ethyl acetate (50:30:20) and purified fractions (Table 3) was obtained for bioassay (Chen and Dai, 2016; Stock and Rice, 1974). Purified fractions of *Solanum nigrum* plant extracts were tested on the insect/pest red flour beetle *Tribolium castaneum* (Najafabadi et al., 2014). For this purpose, adult insect of *Tribolium castaneum* of order Coleoptera and family Tenebrionidae were collected from the food grain store houses available in local market of Bhopal (M.P.). The beetles were reared on healthy cereal grain of wheat *Triticum aestivum* in glass jars (Fig. 3). One thousand insects were released in 1 Kg of wheat grains capped with muslin cloth for ventilation. Culture were maintained in laboratory, under controlled temperature (28±2˚C), relative humidity (75±5% RH) and a photoperiod of 12:12 (L:D) hrs. Then, insects were reared in glass jars on wheat grain and each time early age beetles were used for the experiments. In bio-assay protocol, Group I was served as Control in which insects were reared in maintained laboratory conditions in glass jars of standard size and no treatment was given to the insects of this group. Group II was served as Vehicle Control, in which, insects were reared in glass jars with vehicle solvent (1 ml) that was used for extraction of plant extract, to observe the consequences of vehicle solvent on animals. Group III was served as Treated, in which insects were reared by treating it with different concentration of plant extract in increasing order from 10% (100mg/1ml) to 100% (550mg/1ml), to monitor the insecticidal efficacy of different extracts of each plant. Group IV was served as Standard, in which insects were reared by treating it with standard reference drug Parad Tikdi by dissolving 500mg tablet in distilled water and concentration was maintained 100%, to observe efficacy of standard drug with the comparison of herbal extracts. For the efficacy of herbal extracts, purified fractions of the each plant extracts were tested on the insect/pest red flour beetle Tribolium castaneum (Herbst) for insecticidal activity and three replicates were placed. Mortality in Tribolium castaneum was recorded after 24 hr in the presence and absence of plant extracts, separately (Fig. 4 a, b). Finally, LC_{50} values were calculated according to the methods of Finney (1971). The results were compared with the help of PROBIT analysis, Standard deviation; Standard Error and significant value (p value) were noted down during experiments (Fig. 5 and 6).

RESULTS AND DISCUSSION

In the present study, the percentage yield of the extract of *Solanum nigrum* whole plant was reported to be 1.67% in benzene solvent (Table 2). Similarly, Abbas et al. (2014) have reported percentage yield of *Solanum nigrum* plant extract maximum in water followed by methanol. In further study, crude extracts of *Solanum nigrum* was separated and purified by thin layer and column chromatography. TLC of the extract was done by applying solvent system viz. Petroleum ether: toluene: ethyl acetate (50:30:20) and 4 spots were obtained with Rf value 0.12, 0.40, 0.48 and 0.89. Similar extract was again tested for TLC with another solvent system chloroform: methanol (50:50) and 4 spots were measured with Rf values 0.59, 0.68, 0.78 and 0.90 (Table 3 and Fig 2 a, b, c). Similarly, Khanam and Sultana (2012) have also reported thin layer and column chromatography with different solvent systems viz. petroleum ether: ethyl acetate (70:30), petroleum ether: methylene chloride (50:20) and petroleum ether: chloroform and methanol (60:30:10) and obtained various fractions. Devi (2016) have also discussed thin layer and column chromatography of extract of *Solanum xanthocarpum* using several solvent systems including petroleum ether: methanol (1:1) and petroleum ether: chloroform and methanol (50:20:10) and 7-8 spots were observed. Prathap (2005) has obtained many fractions from the methanolic extract of *Solanum nigrum* by using different solvent systems viz. chloroform: methanol (95:05), toluene: ethyl acetate: chloroform; formic acid (4.5: 5: 6: 1), acetone: water: conc. Ammonia (90: 7: 3), chloroform: acetone: diethyl amine (5:4:1), toluene: ethyl acetate: diethyl amine (7:2:1) and different visualizing reagents viz. Anisaldehyde-H_2SO_4,Vanillin-H_2SO_4, Iodine, Dragendroff's reagent and number of Rf values were measured viz. 0.12, 0.26, 0.39, 0.5, 0.57, 0.70, 0.88 and 0.90 which are found to be very similar to the Rf value obtained in the present study as shown above.

In the present study, insecticidal activity of whole plant *Solanum nigrum* benzene extract purified fraction was tested against insect *Tribolium castaneum* and it was noticed that benzene 1ml (at 100% concentration) as vehicle solvent, when used alone for testing of its efficacy, benzene was found to be slightly toxic for this insect and 10% (100mg/1ml) to 100% (550mg/1ml) benzene extract of this plant, when applied against this insect, it was noticed that maximum efficacy with 90.00±10.00% mortality of the extract reported in triplicate at 90% concentration (Table 5, Fig. 4a, b and Fig. 5). Similarly, Boussaada et al. (2008) have reported insecticidal activity of aerial parts of Asteraceae family's plant extracts viz. *Mantisalca duriaei, Rhaponticum acaule* and *Scorzonera undulata* against *Tribolium confusum*. They have also observed that *Rhaponticum acaule* was highly active and produced 84% mortality in larvae of *Tribolium castaneum* whereas E_3 and E_4 fraction of *Mantisalca duriaei* produced 43% and 57% mortality after three weeks of treatment. Recently, Ebadollahi et al., 2016. have noticed insecticidal activity of *Melissa officinalis* essential oils against T*ribolium castaneum* insect and the LC_{50} values were assessed by PROBIT analysis as 19.418, 18.418 and 16.159µl/l air after 24, 48 and 72 hrs of exposure time, respectively. In the present study, PROBIT analysis of the bioassay results of the purified fraction of plant extracts was done with the help of Med Calc (2016) Statistical Software version (16.8.4) for the testing of goodness of fit which was assessed by Chi square test, Cox and Snell (1989) R^2 regression and Nagelkerke (1991) R^2 regression analysis.

The bioassay results of benzene extract of *Solanum nigrum* whole plant has shown significant effect ($P<0.0001$) (99%) on mortality of insect *Tribolium castaneum.* Chi square test value was reported 36.42 at degree of freedom (DF=1) and Cox and Snell (1989) R^2 regression analysis value was reported 0.28, that was less than 1 and higher value (0.37) of Nagelkerke (1991) R^2 regression showed the significant effect of extract concentration. The regression coefficient value was noticed to be 0.025, which was higher than the standard error 0.0047, resulting higher values of Wald statistics, that was reported to be significant at $P<0.0001$ level i.e. showing that above mentioned plant extract with increasing concentration was observed to be significantly effective for its insecticidal activities. Moreover, the obtained results while uploading in dose response table at 95% confidence intervals, a sigmoid curve was seen between 0 to 100% concentration levels. Similarly, one decade earlier, Rahman et al., 2007 have reported efficacy of some commonly used insecticides on the red flour beetle Tribolium castaneum (Herbst.). They have noticed the mortality of red flour beetle at 72 hrs after treatment due to malathion and dichlorvos and LC_{50} value by PROBIT analysis along with 95% fiducial limit and chi square value was calculated.

Table 1 : Description of collected plant materials

S. No.	Botanical Name	Family	Part Used	Weight of fresh material	Weight of dried material	Loss in weight
1	*Solanum nigrum*	Solanaceae	Whole plant	850gm	120gm	85.9%

Table 2 : Isolation and extraction of plant material by Soxhletion

S. No.	Plant Material used for Soxhletion	Wt. of powdered material	Solvent used for extraction	Weight of plant extract	% Yield of plant extract
1	*Solanum nigrum* Whole plant	100gm	Benzene	1.67gm	1.67%

Table 3 : Thin Layer Chromatography of *Solanum nigrum* extracts

S. No.	Extract used	Solvent System Used	Spots obtained	Rf Value (cm)	Color characteristics		
					Visible	UV	Iodine
1	Entire plant extract	Petroleum ether: Toluene: Ethyl acetate (50:30:20)	Ss-1	0.12	Brown	Violet	Brown
			Ss-2	0.40	Light brown	Pink	Light brown
			Ss-3	0.48	Pale brown	Light Pink	Brownish green
			Ss-4	0.89	Green	Bluish Pink	Green
2	Entire plant extract	Chloroform: Methanol (50:50)	Ss-1	0.59	Light green	Pink	Brown
			Ss-2	0.68	Brownish green	Bluish Pink	Dark brown
			Ss-3	0.78	Yellow	Viole	Light brown
			Ss-4	0.90	Light yellow	Light pink	Yellow

Table 4 : Column Chromatography of *Solanum nigrum* extracts

S. No.	Extract used	Solvent System Used	Fractions obtained	Color characteristics	Active fractions
1	Entire plant Benzene extract	Petroleum ether: Toluene: Ethyl acetate (50:30:20)	Sn-1	Green	Highly bioactive fraction

Table 5 : PROBIT analysis of *Solanum nigrum* Benzene Extract

Groups	Concentration (per Ml)	Sample size (n)	Percent Mean Mortality (Std. Deviation)	Lethal Concentration (95% confidence interval)		DF	Chi-Square $\chi 2$	Cox & Snell R^2	Nagelkerke R^2	Wald statistics
				LC_{50} (Upper - Lower)	LC_{90} (Upper - Lower)					
Control	-	10	0.00 ±0.00							
Vehicle control (Benzene)	1 ml	10	3.33 ±5.77							
Benzene Extract	10%	10	20.00 ±20.00							
	20%	10	26.67 ±5.77							
	30%	10	30.00 ±10.00							
	40%	10	43.33 ±5.77	51.50 (40.82-62.44)	100.84 (85.01-131.12)	1	36.42 (P<0.01)	0.28	0.37	**30.50** (P<<0.01) Coefficient = 0.025 Std. Error = 0.004
	50%	10	63.33 ±35.12							
	60%	10	56.67 ±20.82							
	70%	10	70.00 ±26.46							
	80%	10	83.33 ±11.55							
	90%	10	90.00 ±10.00							
	100%	10	86.67 ±15.28							
Standard Parad Tikdi	100%	10	6.67 ±11.55							

(n=120; Positive cases=55.83%, Negative cases=44.17%), *DF: Degree freedom; LC: Lethal Concentration;

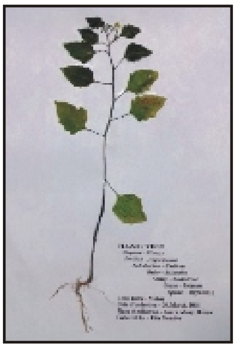

Fig. 1: Taxonomical data sheet of *Solanum nigrum*

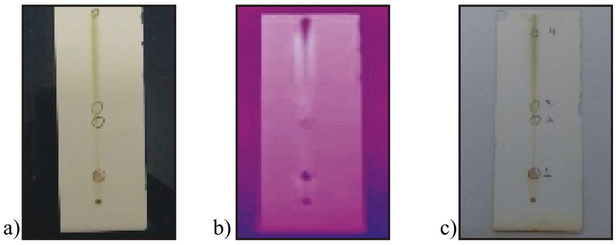

Fig. 2 : Thin layer Chromatography of *Solanum nigrum* plant extract (a) TLC in Visual light (b)TLC in UV light (c) TLC in Iodine chamber

Fig. 3 : Rearing of *Tribolium castaneum* with *Triticum aestivum* wheat grainsin glass jar covered by muslin cloth, respectively

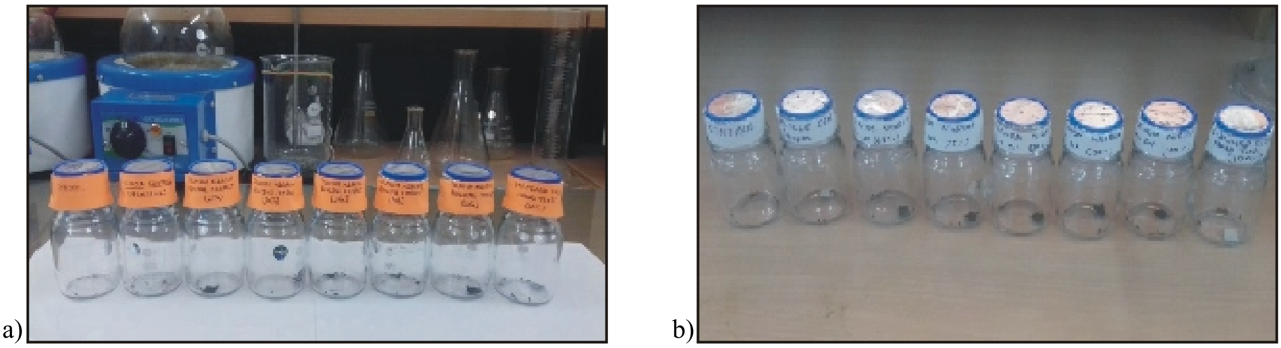

Fig. 4 : (a and b) Bio-pesticidal efficacy of *Solanum nigrum* plant extracts purified fraction on insect *Tribolium castaneum* with concentration range (a)10% - 50%,(b) 60% - 100%)

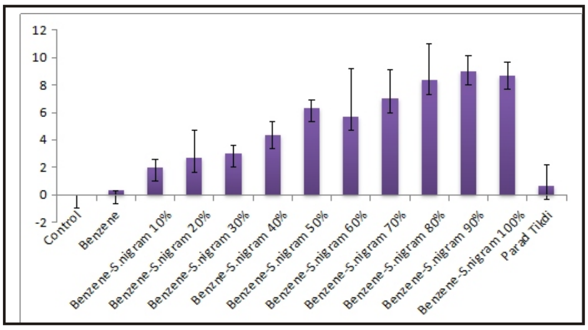

Fig. 5 : Mean Standard graph of *Solanum nigrum* Benzene extract

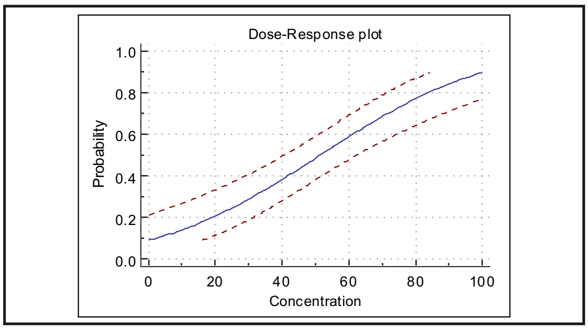

Fig. 6 : Dose response curve of *Solanum nigrum* Benzene extract

CONCLUSION

Finally, it is concluded that *Solanum nigrum* whole plant benzene extract's purified fractions can be an effective means as bio-pesticides of plant origin against insect *Tribolium castaneum*, a well known pest of wheat *Triticum aestivum*. In the present study, the efficacy of benzene extract of the *Solanum nigrum* whole plants was reported against the insect *Tribolium castaneum* for bio-pesticidal activity that is an eco-friendly and economic means of insect management without side effects on non-target organism including man.

ACKNOWLEDGEMENT

Authors are very grateful to UGC New Delhi for awarding UGC/FRPS/start-up grant to Dr. Kapil K. Soni, who has established Pharmacogenomics Laboratory and also thankful to DBT, New Delhi for providing grant through DBT Builder program to Barkatullah University through which corresponding author has developed infrastructure facility in the laboratory.

REFERENCES

Abbas K., Niaz U., Hussain T., Saeed M. Asif, Javaid Z., Indrees A. and Rasool S., 2014. Anti-microbial activity of fruits of *Solanum nigrum* and *Solanum xanthocarpum.* Acta Poloniae Pharmaceutica in Drug Research, **71**(3): 415-421.

Bakkali F., Averback S., Averback D. and Idaomar M., 2008. Biological effects of essential oils. A review. Journal of Food Chemistry and Toxicology, **46**: 464-475.

Boussaada Olfa, Kamel M. B. H., Ammar S., Haouas D., Mighri Z. and Helal A. N., 2008. Insecticidal activity of some Asteraceae plant extracts against *Tribolium confusum.* Bulletin of Insectology, **61** (2): 283-289.

Brimley R.C. and Barrette F.C., 1953. Practical Chromatography. Chapman and Hall Publication, London: 26.

Chen Y. J. and Dai G.H., 2016. Effect of the extract and compound from *Solanum nigrum* Linn on *Tetranychus cinnabarinus.* Journal of Applied Entomology, doi:10.1111/jen.12358.

Cox D.R., and Snell E.J., 1989. The analysis of binary data (2nd, Ed.). London, UK: Chapman and Hall.

Deepthi V. J. and Pradeep K.**,** 2016. Karanja Leaf- A pharmacognostical and phytochemical analysis. Journal of Pharmacognosy and Phytochemistry, **5**(5): 148-151.

Devi Subharani K., 2016. An amazing traditional healer of Manipur *Solanum xanthocarpum* (Leipungkhanga). Intern. J. of Emerging Trends in Science and Technology, **3**(10): 4680-4684.

Dubey S., Sharma P.K., Rajput J., Tomar R. and Baghel A., 2014. Phytochemical analysis of seeds of medicinal plants. Intern. Res. J. Pharm., **5**(2): 102-105.

Ebadollahi A., Parchin R.A. and Farjaminezhad M.; 2016. Phytochemistry, toxicity and feeding inhibitory activity of *Melissa officinalis* L. essential oil against a cosmopolitan insect *Tribolium castaneum* Herbst. Journal Reviews. **35**(4): 77-82.

Farnsworth N.R., 1990. In: Bioactive Compound from Plants. Ciba Foundation Symposium. 1st Edition, John Wiley and Sons, **154**: 2-21.

Finney D.J.,1971. Probit analysis 3rd edition Cambridge Univ. Press. London.

Grainge M. and Ahmed S., 1988. Hand Book of Plants with Pest Control Properties. John Wiley and Sons.

Isman Murray B., 2000. Plant essential oils for pest and disease management. Crop Protection, **19**: 603-608.

Khan F.Z.A., Sagheer M., Hasan M., Saeed S., Ali. K., Gul H.T., 2013. Toxicological and repellent potential of some plant extracts against stored products insect pest *Tribolium castaneum* (Herbst.) (Coleoptera: Tenebrionidae). International Journal of Biosciences, **3**(9): 280-286

Khanam S. and R. Sultana, 2012. Isolation of β-Sitosterol and Stigmasterol as active immunomodulatory constituents from fruits of *Solanum xanthocarpum* (Solanaceae). International Journal of Pharmaceutical Sciences and Research (IJPSR), **3** (4): 1057-1060.

Kulkarni J., Kapse N. and D.K. Kulkarni, 2009. Plant based pesticides for control of H*elicoverpa armigera* on C*ucumis*. Asian Agriculture History, **13** (4): 327-332.

Med Calc; 2016. Statistical Software version 16.8.4 (MedCalc Software bvba, Ostend, Belgium; https://www.medcalc.org).

Nadeem M., Iqbal J., Khattak M.K. and M.A. Shahzad, 2012. Management of *Tribolium castaneum* (Hbst.) (Coleoptera: Tenebrionidae) using Neem (*Azadirachta indica,* A. Juss) and Tumha (*Citrullus colocynthis,* L.). Pakistan Journal of Zoology, 44: 325-331.

Nagelkerke, N., 1991. A note on a general definition of the coefficient of determination. Biometrika, **78**(3): 691-692. doi: 10.2307/2337038

Najafabadi Modarres S.S., Beiramizadeh E. and Zarei R., 2014. Repellency and toxicity of three plants leaves extraction against *Oryzaephilus surinamensis* L. and *Tribolium castaneum* Herbst. J. Bio. And Env. Science, **4**(6): 26-32.

Phillips T.W. and Throne J.E., 2010. Bio-rational approaches to managing stored products. Annual Review of Entomology, **55**:375-397.

Prathap R.V., 2005. Bioactivity guided isolation of actives from some medicinal plants using anti-oxidant models. M. Pharmacy. Dissertation, Govt. College of Pharmacy, Banglore : 1-92.

Rahman M.A., Haque A.H.M.M., Ahmed F., Hossain A.T.M. and Hussain M.F., 2007. Efficacy of some commonly used insecticides on the red flour beetle *Tribolium castaneum* (Herbst.). Int. J. Sustain. Crop. Prod., **2**(5): 08-11.

Ramachandran S., Thiyagarajan S., Alruwaili J. and Hemamalini V., 2014. Demonstration of pre-clinical anti-dermatophytic activity by seed kernels of *Pongamia pinnata* (Linn.). Intern. Journal of Research in Pharmacy and Chemistry, **4**(2): 319-326.

Sagheer M., Hasan M., Latif M.A. and Iqbal J., 2011. Evaluationof some indigenous medicinal plants as a source of toxicant, repellent and growth inhibitors against *Tribolium castaneum* (Herbst.) (Coleoptera: Tenebrionidae). Pakistan Entomologist, **33**: 87-91.

Sagheer M., Hasan M., Rehman H., Khan F.Z.A., Gul H.T., Haidri S.R., 2013. Growth regulatory potential of five indigenous plant extracts against *Tribolium castaneum* (Herbst.) (Coleoptera: Tenebrionidae). International Journal of Biosciences, **3**(12): 50-54.

Sajid Z.I., Anwar F, Ghulam S., Ghulam R., Alkharfy K.M. and Gilani A.H., 2012. Antioxidant, antimicrobial properties and phenolics of different solvent extracts from bark, leaves, and seeds of *Pongamia pinnata* (L.) Pierre. Molecules, **17**, 3917-3932.

Stock, R. and Rice C.B.F.; 1976. Chromatographic methods. Chapman and Hall Publication, London: 376.

Tswett, M.; 1906. Invention of chromatography. Ber. Dtsch. Botan. Ges, 24: 316-323.

Upadhyay R.K. and Ahmad S., 2011. Management strategies for control of stored grain insect pests in farmer stores and public ware houses. World Journal of Agricultural Sciences, **7**: 527-549.