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EVALUATION OF THE EFFICACY OF INDIGENOUS BOTANICAL EXTRACTS AS GROWTH INHIBITORS AGAINST TRIBOLIUM CASTANEUM

Saif-Ud-Din[1*], Shaukat Ali[1], Maisoor ahmed Nafees[2], Muhammad Arshad[3], Salar Ali[4], Nighat Gul[2], Babar Hussain[5]
[1]Department of Environmental Sciences, Karakoram International University, Gilgit, Pakistan
[2]Department of Animal Sciences, Karakoram International University, Gilgit, Pakistan
[3]Department of Agriculture & Food Technology, Karakoram International University, Gilgit, Pakistan
[4]Department of Environmental Sciences, University of Baltistan Skardu, Pakistan
[5]Department of Plant Sciences, Karakoram International University, Gilgit, Pakistan

Article Info

*Corresponding Author
Email: saifuddin@kiu.edu.pk

Abstract

This study was attempted to assess the toxic efficacy of three plants extracts Chenopodium botrys (C.B), Carthamus Tinctorius (C.T), Mentha Royleana (M.R) and their mixed solutions against Tribolium Castaneum in laboratory environments by filter paper impregnation process. Selected concentrations for each mixed plants were 5 %, 2.5 %, 1.2 5%, 0.63 % and 0.315 % which are then transformed into µg/cm2 for more estimations. After the transformation the values perceived as 1273.88 µg/cm2,636.94 µg/cm2, 318.47 µg/cm2, 160.5µg/cm2, 80.25 µg/cm2 µg/cm2 were tested for 24, 48, 72, 96, 120, 144 and 168 hours separately. The percentage mortality for each single and mixed extracts was acquired. Maximum mortality (78.14%) was perceived by the single extract of M. royleana treated with 1273.88µg/cm2. While lowermost mortality (17.14%) was gained by single extract of C. tinctorius treated with 80.25µg/cm2. Single plants extract of M. royleana (66.66 %) decreases the level of alkaline phosphatase and decreases (59.94%) the level of acid phosphatase. It is concluded that the single plant extract of M. royleana is more lethal and active as compared to the other single and mixed extracts.

Keywords
Efficacy, Botanical Extract, Growth Inhibitor, Tribolium Castaneum, Gilgit

1. Introduction

Various studies have conducted worldwide in order to replace the synthetic pesticides with plant extracts due to increasing environment and health related issues. (Khan *et al*., 2016). Various plant extracts have been proven with positive insecticidal action. The plant derivative chemical activates after plants extracts screening and has been used active protectant as an antifeedant and insecticides and usual antifeedant are used to control pest, to evade environmental contamination and to diminish practice of synthetic pesticides (Ali *et al*., 2018).The poisonous effects of plant extracts on insect pests were confirmed in numerous means comprising mortality, growth inhibition, overthrow of reproductive behavior and through their influence on fertility and potency of eggs. (Mulunga *et al*., 2007; Abida *et al*., 2010; Begum *et al.,* 2011; Susana *et al*., 2013). The Store grain damages has been estimated to 10-40% worldwide, while in Pakistan they have been 10-20%. These damages are triggered by insects, microbes and other pests (Khan et al., 2010; Rashid *et al*., 2012) Various plants flourish in rough environmental settings so they have developed a various of defense mechanisms against natural enemies in their evolutionary period. Among these defense mechanisms subtle chemical and morphological mechanisms are more effective. These microbes and pests generally do not cause abrupt death but hinder with their energetic biochemical and physiological functions (Prakash & Rao, 1997).

2. Materiel and Methods

2.1 Collection and Preparation of Plant Materials

Fresh leaves of the plants Chenopodium botry (C.B), Carthamus tincterious (C.T) and Mentha roleyana (M.R**)** were collected from different locations of District Gilgit. The sample plants were washed with water after drying in shade samples were smashed to make a fine powder. The plants extracts were synthesized according to the method described by Valsaraj et al. In 300 mL of Methanol 99% (w/v) ratio 100 gram of powder was mixed at room temperature. The mixtures were then mixed for 45 minutes in an ultrasonic bath at persistent temperature of 25 °C, allowed to stand for 72 hours and shacked numerous times at certain intervals. Single and Combined solution of C. botrys + C. tinctorius; C. tinctorius +M. royleana**;** C. botrys +M. royleana; C. botrys +C. tinctorius +M. royleana were made. Finally, different concentration including 5, 2.5, 1.25, 0.63 and 0.31% of each extract were made. With the help of charless equation the dilutions from these stock solutions were prepared.

$$C1\ V1 = C2\ V2$$

2.2 Insect Culture

On a medium of wheat flour red floor beetles Tribolium castaneum were cultured which were collected from stored grains. This culture was maintained in the laboratory at persistent temperature 29±1ºC and 65±5% relative humidity (RH).T.castaneum was raised on wheat flour in the water quality laboratory Department of Biological Sciences, Karakorum International

University Gilgit. Tribolium castaneum adults were retained in glass jars (250 mL) comprising wheat flour and 5% yeast; jars were enclosed with black fabric to allow air and darkness at the top. The insects were raised in the laboratory at 25°±2°C and 50±5% RH (Ali *et al*., 2018; Gul *et al*., 2019).

2.3 Toxicity Determination

Entire extracts were tested by filter paper impregnation technique (Azmi, 2004). For this determination, seven sets of Petri dishes of 2.5cm were reserved, washed, air dried and autoclaved. Five petri dishes were labelled for dose however two were set aside as control and Methanol. On the bottom of petri dish filter paper was placed and with the help of pipette five diverse concentrations were spreaded on filter paper. Similar age and sized 10 adults were introduced in each petri dish separately. Mortality was observed after 24 hours of treatment (Ali *et al*., 2018; Gul *et al*., 2019).

2.4 *Determination of Lethal Value*

In order to determine LC_{50} of seven extracts graph between dose and mortality was plotted on logprobit. In this method, we initiated the lethal concentrations at which 50% of the tested population was killed.

2.5 Biochemical Estimation.

For the preparation of homogenate, 100 adult beetles of similar size and age of T. castaneum were tested by LC_{50} of all single and mixed extracts. A control and methanol were also retained to determine the environmental and methanol effect for the estimation of acid and alkaline phosphatase a control and methanol were also kept. Level of alkaline phosphatase was resoluted by the colorimetric kit method of Randox Cat No. 307, which is established upon the process of Rec (1972). Level of acid phosphatase was resoluted by the colorimetric kit technique of Randox Cat No. 1011, which is established upon the process of Hillmann (1971).

3. Result and Discussion

In the present study C. botrys, C. tinctorius and M. roleyana and their mix extracts were tested against T. Castaneum by the filter paper impregnation technique. The percentage mortality values to each particular and mixed extracts were gained. Five concentrations (5%, 2.5%, 1.25%, 0.63% and 0.315%) were treated and the mortality was checked at 24, 48, 72, 96, 120, 144 and 168 hours post treatment. When T. castaneum was tested with single extract of C.B LC_{50} was perceived (659.2 ug/cm^2), while C.T gained LC_{50} (682.66 ug/cm^2), whereas M.R showed highest mortality and LC_{50} (437.92 ug/cm^2). Whereas, T. castaneum was tested with mixed derivatives of C.B + C.T the LC_{50} obtained (552.98 ug/cm^2), while C.T +M.R LC_{50} (602.55 ug/cm^2), C.B +M.R (LC_{50} 501.44 ug/cm^2), and the LC_{50} of C.B +C.T + M.R obtained (466.56 ug/cm^2). There is an improved concern among researchers to study the bioactivity of plant derivatives against insect pests (Dubey *et al*., 2008; Benzi *et al*., 2009). In an earlier study Saeed *et al.* (2016) received better ability of neem, the black pepper as long as high mortalities at entirely doses. Earlier outcomes of Shayesteh

and Ashouri (2010) also reinforced these results, investigated the effect of four powdered spices viz, black pepper, chili pepper, cinnamon and turmeric as repellents against adults of R. dominica F., S. granarius (L.) and T. castaneum (Herbst) and found all of these plant species to be highest activity at the highest dose (2.5%) and after interval (24 hours).Biological activity of leaves and fruits extract of the African shrub Boscia senegalensis was inspected against Callosobruchus maculatus and mixed the extracts with cowpea at 2-4% (w/w), fresh ground fruits and leaves caused 80-100% mortality in Callosobruchus maculatus (F.) adults (Seck *et al*., 1993).

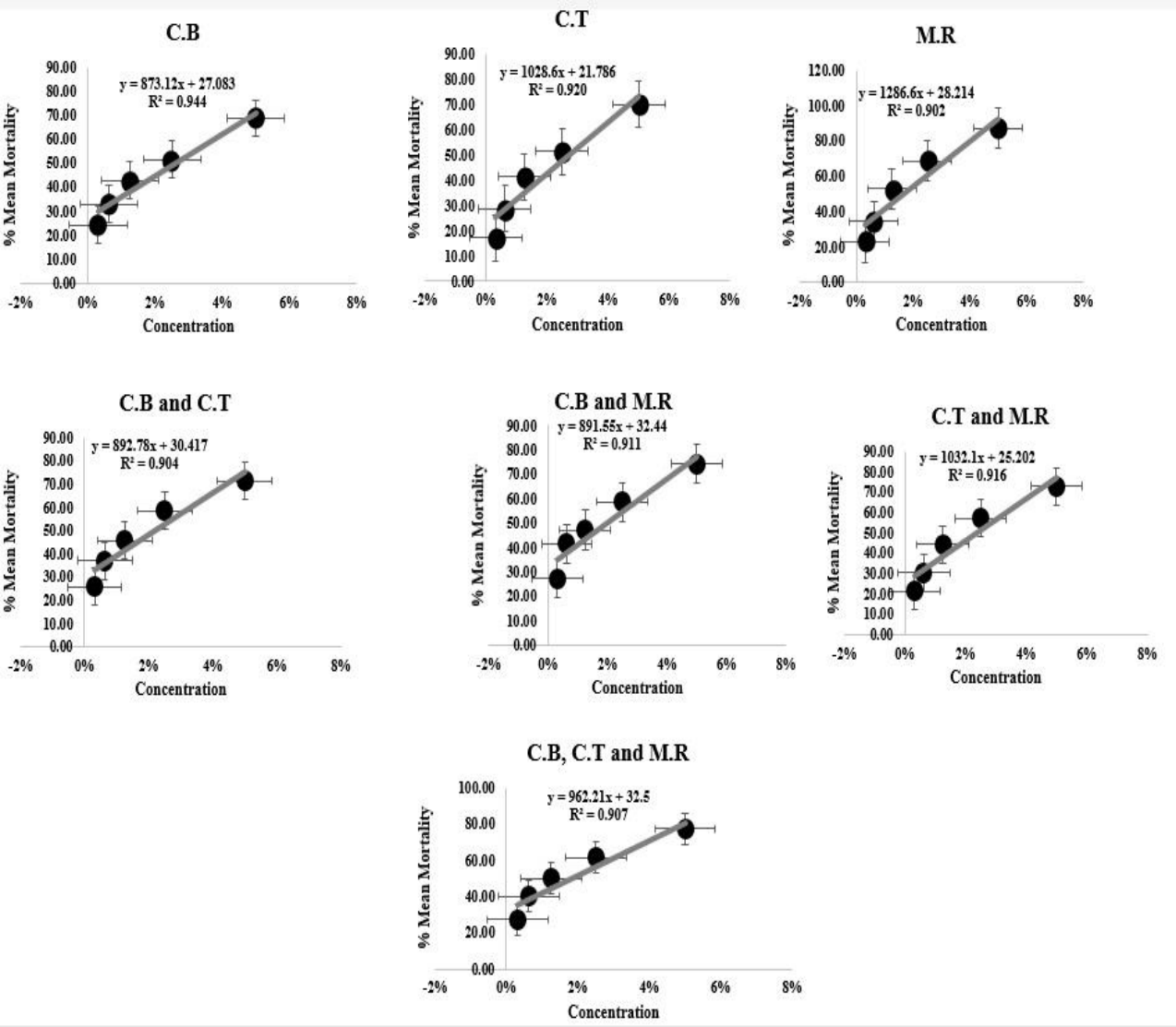

Figure 1: Mean mortality of single and mixedextract of plant against T. castaneum.

Table 1: LC50 and mean mortality of single and combined extract of plants against T. castaneum

Conc ug/cm2	Chenopodium botrys (C.B)	Carthamus tinctorius (C.T)	Mentha royleana (M.R)	C.B+C.T	C.T+M.R	C.B+M.R	C.B+C.T+M.R
1273.88	68.57±7.41 ^{C}	70±6.84 ^{CD}	87.14±7.01 ^{A}	71.42±6.53 ^{CD}	72.85±5.32 ^{BCD}	74.28±6.71 ^{BC}	77.14±6.55 ^{B}
636.94	51.42±5.35 ^{C}	51.42±5.32 ^{C}	68.57±5.47 ^{A}	58.57±5.71 ^{B}	57±4.90 ^{B}	58.57±4.74 ^{B}	61.42±4.82 ^{B}
318.47	42.85±6.03 ^{C}	41.42±4.71 ^{C}	52.85±6.08 ^{A}	45.71±4.07 ^{BC}	44.28±4.73 ^{BC}	47.14^{ABC}±5.76	50±3.41 ^{AB}
160.5	32.85±4.92 ^{CD}	28.57±4.64 ^{C}	34.28±3.99 ^{BCD}	37.14±3.04 ^{ABC}	30.42±3.12 ^{CD}	41.42^{A}±3.84	40±3.39 ^{AB}
80.25	24.28±4.37 ^{AB}	17.14±3.23 ^{C}	22.85±3.17 ^{AB}	25.71±2.98 ^{AB}	21.42±2.46 ^{BC}	27.14±2.09 ^{A}	27.14±2.70 ^{A}
LC50	**659.2**	**682.66**	**437.92**	**552.98**	**602.55**	**501.44**	**466.58**

Means in each row followed by the same letter are not significantly different at LSD test (P = 0.05)

Table 2: Activity of Acid Phosphatase in *T.* Castaneum after 24 hour of treatment

Extracts	Treatment	Mean µ/L	S.D. (±)	S.E. (±)	% Inhibition
Chenopodium botry (C.B)	**Control**	6.687	1.7	0.82	
	Treated	4.458	1.2	0.67	24.93
Carthamus tincterious (C.T)	**Control**	5.201	1.4	0.57	
	Treated	2.972	1.1	0.68	19.67
Mentha roleyana (M.R)	**Control**	4.458	1.6	0.91	
	Treated	1.486	0.7	0.25	59.94
C.B+C.T	**Control**	5.944	1.2	0.97	
	Treated	3.715	1.1	0.97	15.56

C.T+M.R	**Control**	5.201	1.8	0.89	
	Treated	2.229	1.2	0.76	39.75
C.B+M.R	**Control**	3.715	1.6	0.69	
	Treated	2.229	1.1	0.58	21.44
C.B+C.T+M.R	**Control**	5.944	1.4	0.98	
	Treated	2.972	1.2	0.64	33.31

Table 3: Activity of Alkaline Phosphatase in T. Castaneum after 24 hour of treatment

Extracts	**Treatment**	**Mean µ/L**	**S.D. (±)**	**S.E. (±)**	**% Inhibition**
Chenopodium botry (C.B)	**Control**	22.08	5.8	3.2	
	Treated	16.56	4.2	3.1	14.28
Carthamus tincterious (C.T)	**Control**	16.56	3.7	2.5	
	Treated	11.04	3.4	1.9	20
Mentha roleyana (M.R)	**Control**	11.04	4.6	2.3	
	Treated	2.76	3.9	1.6	66.66
C.B+C.T	**Control**	13.8	4.2	2.4	
	Treated	8.28	3.1	1.1	25
C.T+M.R	**Control**	19.32	4.9	1.9	
	Treated	13.8	2.7	2.1	16.65
C.B+M.R	**Control**	16.56	3.6	1.8	
	Treated	8.28	3.2	1.4	40
C.B+C.T+M.R	**Control**	24.84	5.4	3.7	
	Treated	16.56	4.6	2.4	14.28

When adult's beetles of Tribolium Castaneum were tested by using Clerodendrum inerm and cyhalothrin both compound decreases the acid phosphatase level very little volume. (Rizvi *et al*., 2001). Similar outcomes were obtained when tested similar pesticides against the adult beetle of Rhizopertha dominica, for acid phosphatase level (Azmi, 2004). Whereas in our research LC50 of CB, CT, MR, mixed extracts of CB and CT, mixed of CT and MR, mixed of CB and MR and combined CB, CT and MR were tested for Acid Phosphatase inhibition in relating with toxicological effects of tested extracts on Acid Phosphatase level of T. castaneum calculated after 24 hours of treatment. CB derivative reduces 24.93%, CT reduces 19.67%, and MR decreases 59.94.66%, whereas mixed derivative of CB and CT reduces 15.56%, mixed of CT and MR reduces 39.75% and mixed of CB and MR reduces 21.44%, however mixed of CB, CT and MR reduces 33.31% in Acid Phosphatase level. Adults of Sitophilus orzyzae were tested by using neem leaves derivative decreases alkaline phosphatase level i.e., 18.39% (Ahmad *et al.*, 2000) While in the current investigation LC50 of CB, CT, MR, mixed extracts of CB and CT, mixed of CT and MR, mixed of CB and MR and combined CB, CT and MR were treated for Alkaline Phosphatase inhibition in linking with toxicological effects of tested extracts on Alkaline Phosphatase contents of T. castaneum calculated later on 24 hours of treatment. CB derivative reduces 14.28%, CT reduces 20%, and MR decreases 66.66%, whereas mixed derivative of CB and CT reduces 25%, mixed of CT and MR reduces 16.65% and mixed of CB and MR reduces 40%, however mixed of CB, CT and MR reduces 14.28% in Alkaline Phosphatase content. In earlier research Sitophilus oryzae was tested by using cyfluthrin both the enzymes Acid and alkaline phosphatase level were reducing in their actions by 35.08% and 26.49% respectively (Rizwan *et al*., 2000). Decrease little amount of acid and alkaline phosphatase level was found for the period of investigation of JHA (Methoprene on alstoid ZR-515) to M. domestica larvae (Qureshi *et al*., 1983). The enzyme activity was decreased due to digestion of tested extracts, which decreased gut enzymes such as acid and alkaline phosphatase. Proposed Results suggest that these enzymes are the more sensitive to pesticide exposure (Wu and Lam, 1997; Diamantino *et al*., 2001; de Almeida *et al*., 2014; Ottaviani, 2014).

4. Conclusion

It is concluded that all the plant products affected mortality and reduces the Acid and Alkaline phosphatase level, in T. Castaneum however; single extracts M. roleyana was caused high mortality and more shrinks Acid and Alkaline phosphatase level as compare to mixed extracts.

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