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Mode and Mechanism of Action of Herbicidal Metabolites from *Phoma* sp. FGCC#54 Effective against Prominent Weeds of Central India

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Abstract: *Weed management plays a crucial role in maintaining economy of a developing country. Losses incurred due to the loathsome plants- weeds are incredibly severe and constitute an indispensable factor determining crop productivity and agricultural sustainability. Weed management primarily aims in curbing weed population to economic and lethal thresholds. Biological agents and their metabolites prove to be effectual for weed management due to their cost-effective and eco-friendly properties over hazardous, harmful, traditional chemicals. Herbicides exhibit various mode and mechanism of actions, against the target weeds, which becomes the thrust area of research. In the present study, phytotoxic metabolites of fungus Phoma herbarum FGCC#54- Phytotoxin I and II produced remarkable effects on biological contents of four test weed plants. In addition to this electrolyte leakage studies indicate a possible role of these metabolites in mitigating weed plants' populations.*

Introduction

Weeds are unwanted plant species competing with other plants for water, nutrients and light leading to reduction in crop yield and productivity (Abbas and Duke 1995). They are notorious and ethically obnoxious having inhibitory effect on crops and other plants and also cause various health hazards (Quereshi et al., 2010; Gadermaier et al., 2014). Therefore, their eradication from the infested areas becomes quintessential. Various traditional and chemical methods have been employed for eradication of weeds (Green, 2011). Microbial metabolites due to their phytotoxic properties always attract researchers for weed management using biological agents (Fischer and Bellus 1983; Duke,1986). Microbial secondary metabolites with unique mode and mechanism of action provide ecofriendly alternatives to commercial herbicides (Kenfield et al. 1988). *Parthenium hysterophorus*, *Lantana camara*, *Hyptis suaveolens* and *Sida acuta* are important weeds of Central India, causing great menace to non-cropped lands making the area unfit for either cattle grazing or residing by human beings.

Isolating natural products from microorganisms as secondary metabolites and screening for herbicidal activity may offer new templates for developing novel herbicides with desired mode and mechanisms of actions (Mullner *et al*., 1993). Commercial herbicides combined attack only about 15 molecular sites of action (Devine *et al*., 1993; Duke, 1990). Phytotoxins from biocontrol fungi involve an array of different 'modes of action' which might result in complete seed germination inhibition and/or encumber major metabolic pathways leading to complete death of plants ((Kenfield et al. 1988). Many commercially available herbicides originally derived from microbial sources, attack the same molecular site, different from any of the herbicides. Microbial phytotoxins are largely water soluble, non-halogenated compounds, whose molecular sites of action differ in almost every case from the sites of commercial herbicides (Duke *et al*., 1991). Microbial phytotoxins are used as tools for envisaging new molecular sites of action not discovered by the traditional or commercial herbicides (Cutler, 1991). Microbial products thus are natural products less detrimental to the environment and less toxic to human than synthetic compounds. Due to their isolation from host specific plant pathogens and weed hosts, microbial products from plant pathogens tend to be more selective than synthetic compounds (Saxena and Pandey, 2001). Bailey et al. (2011, 2013) studied the effect of fertilizers on the efficacy of the bioherbicide, *Phoma macrostoma*, for controlling dandelions in turfgrass. Various scientists are exploring various fungal genera for their potential as biological weed control candidates.

In the present study two earlier reported phytotoxins from *Phoma herbarum* FGCC#54 have been explored for their effects on biological activities

(effects on chlorophyll and protein contents) and electrolyte leakages on application on test weeds.

Materials and methods

Estimation of chlorophyll

1 gm of fresh leaves were homogenized with excess ethanol in a pestle and mortar and centrifuged at 8000 rpm for 2 min with 80% ethanol, supernatant was taken in other flask and diluted 10 times with ethanol. Absorbance of extract at 645 and 663 nm was measured for the determination of chlorophyll a, b and total chlorophyll (Arnon, 1949). The chlorophyll content was determined on a fresh weight basis employing the following formula:

Total chlorophyll (mg/ml)

$$= \frac{20.2\ A_{645} + 8.02A_{663}}{a \times 1000 \times w} \times V$$

Or

$$= \frac{27.8\ A_{652} \times V}{a \times 1000 \times w}$$

$$\textbf{Chl a (mg/ml)} = \frac{12.7\ A_{663} - 2.69A_{645}}{a \times 1000 \times w} \times V$$

$$\textbf{Chl b (mg/ml)} = \frac{22.9\ A_{645} - 4.68A_{663}}{a \times 1000 \times w} \times V$$

Where,

a = length a light path in the cell (usually 1cm).

v = volume of the extract in ml.

w = fresh weight of the sample in gms.

Estimation of proteins

Diseased as well as healthy tissues of target weed were macerated in a mortar with pestle and filtered with filter paper. Extracts were centrifuged at 2000 rpm for 20 min and discarded in 5% TCA at 0^0C for 15 min in an ice bath, to precipitate the proteins and again centrifuged, supernatant was discarded This process was repeated twice and the pellets were re-extracted with absolute ethanol and supernatant was discarded It was again processed with hot ethanol ether mixture. This pellet contains protein and nucleic acid (Osborne, 1962).

To determine total protein method suggested by Lowry *et al*. (1951) was followed. The amount of protein in the samples was calculated with a standard curve which was prepared earlier using bovine serum albumin (Lowry *et al*., 1951).

Electrolyte Leakage Studies: Target weed leaves were surface sterilized with 0.02% NaOCl solution for 5 minutes, then rinsed thrice in sterile distilled water, 5mm diameter discs of leaves were cut with the help of a sterilized cutter. The leaf discs (50 mg; 8 to 10 discs) were placed in a tube containing 1 ml of test liquids, namely the culture filtrate or a solution prepared by dissolving the crude organic extract in Distilled water. The discs were infiltrated with the toxin, rinsed with double distilled water several times and leached against 20 ml of double distilled water for 1.5 hr. The conductivity of this latter solution was measured with a conductivity meter. Leaf discs infiltrated with double distilled water were used as controls. Each test was performed in triplicate (Parisi *et al*., 1993).

Results and discussion

Effect on chlorophyll contents

Percentage reduction in chlorophyll contents of test weed leaves by detached leaf bioassay on treatment with TLC purified phytotoxin I (Quereshi et al., 2011) of the test fungal strain is represented in the **Table 1).** Results show that maximum percentage reduction in chlorophyll occurs after 48hpt, followed by 36 and 24 hpt as determined by Detached leaf bioassay. Minimum reduction was observed after 12 hpt. In general total chlorophyll was affected the most while this was followed by chlorophyll b and chlorophyll a for *Parthenium*, *Hyptis* and *Sida*. While, chlorophyll b was affected the most followed by chlorophyll b and total chlorophyll in case of *Lantana*. Similarly, **Table 2** depicts detached leaf bioassay results for TLC purified Phytotoxin II (Kalam et al., 2014). Results are consistent with those obtained for TLC purified Phytotoxin I. Thapar *et al*., 2002 recorded significant reduction in biological contents of *Parthenium* treated with CFCF of *Curvularia lunata*. Singh, 2007 also reported reduction in chlorophyll content of prominent weeds of Madhya Pradesh on treatment with CFCF of *Fusarium* spp. Abbas *et al*., 1992 also recorded 25-78% reduction in cholorophyll content in Jimson weed tissues treated with fumonisin. Chlorosis and necrosis caused the loss of chlorophyll from leaves. Depletion of chlorophyll is due to phytotoxic effects of the secondary metabolite obtained from *Phoma* sp. FGCC#54. Destruction of the two chlorophyll affects both the photosystem of photosynthesis, since photosystem I is associated with chlorophyll a and Photosystem II with chlorophyll b. Similar results were obtained by other workers while working with

fumonisins (Duke & Duke, 1997). The action of FBI toxin on jimson weed is identical to herbicide action (Abbas *et al*., 1992). The decrease in chlorophyll might be due to rapid destruction of plasma membrane. They cause the photodynamic porphyrin intermediate, proto-porphyrin IX, to accumulate in the plasma membrane via a complex mechanism, resulting in plasma membrane lipid peroxidation (Duke *et al*., 1991). Rapid loss of plasma membrane integrity is consistent with the rapid cellular leakage detected with phytotoxins (Abbas *et al*., 1992, Duke *et al*., 1991).

Effect on protein contents

Protein present in plant tissues has to be separated by precipitation and clarified from other interfering substance prior to their estimation. Percentage reduction in protein contents of test weed leaves by detached leaf bioassay on treatment with TLC purified phytotoxin I of the test fungal strain is represented in the **Table 3.** Results show that maximum percentage reduction in chlorophyll occurs after 48hpt, followed by 36 and 24 hpt. Minimum reduction was observed after 12 hpt. Percentage reduction in protein contents of target weed leaves by detached leaf bioassay on treatment with TLC purified phytotoxin II of the test fungal strain is represented in the **Table 4.**

On contrary to detached leaf bioassay, other bioassay results are in agreement to present results. A drastic reduction in protein content (82.6%) was reported by Pandey *et al*., 2007 by treating *Hyptis* shoots with Cell Free Culture Filtrates of *Helminthosporium* sp.FGCC#74. Pandey *et al*., 2005 reported a maximum reduction in protein contents (50%) in case of shoots of *Lantana camara* treated with Cell free Culture Filtrate of *Aspergillus flavus* FGCC#603 followed by *A. nidulans* FGCC#601 obtained from 21 days old fermented broth. Similar results of reduction of protein content due to phytotoxin treatment of *Fusarium* spp. on prominent weeds of Madhya Pradesh have been reported by Singh, 2007.

Electrolyte leakage studies

Considerable electrolyte leakage was obtained after different incubation hours as evident from data presented in **Table 5 & 6**. Maximum electrolyte leakage was observed after 3hpt, followed by 2hpt. Mild loss was seen after 1hpt for both the phytotoxins.

Mohanraj *et al*. (2003) studied pathogen toxin-induced electrolyte leakage in sugarcane. *Colletotrichum falcatum* phytotoxins induced necrotic foliar lesions in susceptible sugarcane varieties followed by disintegration and drying of tissues. One way in which the phytotoxins of some plant-pathogenic fungi act is by disrupting the membrane function of the host, resulting in enhanced electrolyte leakage from affected tissues and contributing to symptom development (Scheffer & Livingston, 1980). Cell free extracts of plant pathogens or elicitors induce electrolyte leakage in host tissues in the absence of the pathogen (Rogers *et al*., 1988). Several workers have studied electrolyte leakage in a ariety of plants (Duke *et al*, 1983; Peever and Higgins, 1989;Whitlow *et al*., 1992). Parisi *et al*., 1993 carried out electrolyte leakage bioassay with the phytotoxin Mellein obtained from culture fluids of *Phoma tracheiphila*.

The gross physiological effects of a toxin if known, the molecular site of action frequently provide the basis for development of rapid and sensitive bioassays. Sensitivity may be increased if the molecular site can be exposed directly to toxin. If a specific enzyme or organelle is found to interact with a toxin, the response may be quantitative as well as sensitive.

Toxins might bind with enzyme's active site like Phaseolotoxin inhibits Ornithine carbamoyltransferase (OCT) from various sources (Mitchell, 1979). The toxins might have direct effects on isolated organelles like chloroplasts (Yoder, 1980). A more common effect of toxin action is damage to plasmalemma, thus the toxin have plasmalemma as the primary site of action (Rudolph, 1976).

Not much information regarding the mode and mechanism of action of natural phytotoxins is available. Some workers although have studied in this respect (Rao, 2000). Role of pathogen produced toxins has been extensively reviewed (Abbas and Boyette, 1992; Abbas *et al*., 1995). The presence of a toxin in the Culture Filtrate of a pathogen does not necessarily implicate its causative role in the disease. Pathogen- produced toxins are classified as primary, secondary or non-determinants of pathogenicity. Toxins not required for the induction of the disease syndrome are secondary determinants. Since these toxins determine the degree of disease development they are considered as virulence factors. Toxins having no role in disease initiation or virulence are non-determinants.

Dayan *et al.* (2002) reported an isolate of *Fusarium solani* NRRL 18883 which produces the natural phytotoxin 2, 5-anhydro-D-glucitol, that was highly phytotoxic on several species. The ability of a pathogen to infect and invade a compatible host may be facilitated by the production of toxins that induce cell death in the proximity of the invading organism (Dangl and Jones, 2001). Studies on the

pathogenicity of fungi have resulted in the isolation and identification of many potent natural phytotoxins from genus *Fusarium*- Fumonisins, Moniliformin, Fusaric Acid and Tricothecenes (Boyette *et al*., 1993; Jin *et al*., 1996).These natural toxins play important roles in inhibiting the physiological processes in cells surrounding the point of infection, enabling the spread of the disease (Feys and Parker, 2000).

Thus, the present work deals, with a critical evaluation of the changes in biological contents (chlorophyll and protein) and electrolyte leakage encountered on treatment of the four obnoxious test weeds, with the two phytotoxins from *Phoma herbarum* FGCC#54. These phytotoxic metabolites exhibit various intrinsic limitations common to all biological agents. Henceforth, further stringent studies need to be vigorously undertaken through field trials for their wide acceptability and practical use.

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Table 1: Reduction in chlorophyll content due to TLC purified phytotoxins in test weeds (Phytotoxin I)

Incubation hours (hpt)	Weeds	Control a	Control b	Chl a	Chl b	Total chl
12	*Parthenium hysterophorus*	0.00±0.00	0.00±0.00	24.45±0.64	30.65±0.53	33.34±0.63
	Lantana camara	0.00±0.00	0.00±0.00	31.99±0.65	29.48±0.50	27.71±0.59
	Hyptis suaveolens	0.00±0.00	0.00±0.00	18.73±0.76	23.41±0.65	25.43±0.62
	Sida acuta	0.00±0.00	0.00±0.00	15.33±0.44	19.34±0.53	22.34±0.54
24	*Parthenium hysterophorus*	0.00±0.00	0.00±0.00	27.76±0.56	33.43±0.56	38.39±0.83
	Lantana camara	0.00±0.00	0.00±0.00	36.43±0.45	32.24±0.63	28.52±0.59
	Hyptis suaveolens	0.00±0.00	0.00±0.00	25.12±0.82	23.55±0.49	31.18±0.56
	Sida acuta	0.00±0.00	0.00±0.00	17.75±0.56	22.37±0.70	29.53±0.73
36	*Parthenium hysterophorus*	0.00±0.00	0.00±0.00	68.83±0.60	70.61±0.61	75.44±0.56
	Lantana camara	0.00±0.00	0.00±0.00	71.20±0.66	69.38±0.42	65.37±0.63
	Hyptis suaveolens	0.00±0.00	0.00±0.00	33.37±0.45	35.46±0.55	47.21±0.58
	Sida acuta	0.00±0.00	0.00±0.00	32.17±0.62	34.61±0.56	44.56±0.74
48	*Parthenium hysterophorus*	0.00±0.00	0.00±0.00	74.63±0.44	79.39±0.36	83.47±0.69
	Lantana camara	0.00±0.00	0.00±0.00	73.95±0.82	71.19±0.66	67.71±0.60
	Hyptis suaveolens	0.00±0.00	0.00±0.00	39.62±0.41	47.46±0.64	59.18±0.56
	Sida acuta	0.00±0.00	0.00±0.00	37.66±0.70	43.28±0.60	53.28±0.58
SEm±		0	0	0.64	0.46	0.39
$LSD_{5\%}$		0	0	1.85	1.53	1.46

Values are Means ± SEM of three observations; Control a- Unmetabolised growth medium; Control b- Sterilized Distilled Water; Amount of Toxin employed=5 ml/leaf; RH-85% PDR- 0-0.99= slight curling & wilting; 1-1.99= 1-20%LAD; 2-2.99=21-40% LAD; 3-3.99= 41-60% LAD; 4-4.99 = 61-80%LAD; 5 = 81 100%LAD (leaf area damage)

Table 2: Reduction in protein content due to TLC purified phytotoxins in test weeds (Phytotoxin I)

Incubation hours (hpt)	Weeds	Control a	Control b	% Reduction in protein
12	*Parthenium hysterophorus*	0.00±0.00	0.00±0.00	66.66±1.20
	Lantana camara	0.00±0.00	0.00±0.00	63.66±1.85
	Hyptis suaveolens	0.00±0.00	0.00±0.00	59.33±0.88
	Sida acuta	0.00±0.00	0.00±0.00	52.00±1.53
24	*Parthenium hysterophorus*	0.00±0.00	0.00±0.00	73.00±1.15
	Lantana camara	0.00±0.00	0.00±0.00	70.33±1.45
	Hyptis suaveolens	0.00±0.00	0.00±0.00	64.00±1.15
	Sida acuta	0.00±0.00	0.00±0.00	60.33±1.45
36	*Parthenium hysterophorus*	0.00±0.00	0.00±0.00	80.00±1.15

	Lantana camara	0.00±0.00	0.00±0.00	76.66±1.20
	Hyptis suaveolens	0.00±0.00	0.00±0.00	72.66±1.45
	Sida acuta	0.00±0.00	0.00±0.00	67.00±1.52
48	*Parthenium hysterophorus*	0.00±0.00	0.00±0.00	84.66±0.88
	Lantana camara	0.00±0.00	0.00±0.00	81.33±0.88
	Hyptis suaveolens	0.00±0.00	0.00±0.00	76.66±1.76
	Sida acuta	0.00±0.00	0.00±0.00	75.00±1.15
SEm±		0	0	0.49
$LSD_{5\%}$		0	0	1.37

Values are Means ± SEM of three observations; Control a- Unmetabolised growth medium; Control b- Sterilized Distilled Water; Amount of Toxin employed=2 ml/leaf ; RH-85% PDR- 0-0.99= slight curling & wilting; 1-1.99= 1-20%LAD; 2-2.99=21-40% LAD; 3-3.99= 41-60% LAD; 4-4.99 = 61-80%LAD; 5 = 81 100%LAD (leaf area damage)

Table 3: Reduction in chlorophyll content due to TLC purified phytotoxins in test weeds (Phytotoxin II)

Incubation hours (hpt)	Weeds	Control a	Control b	Chl a	Chl b	Total chl
12	*Parthenium hysterophorus*	0.00±0.00	0.00±0.00	23.25±0.65	29.60±0.45	32.19±0.53
	Lantana camara	0.00±0.00	0.00±0.00	31.23±0.66	28.48±0.43	26.48±0.55
	Hyptis suaveolens	0.00±0.00	0.00±0.00	18.25±0.55	22.60±0.53	24.27±0.56
	Sida acuta	0.00±0.00	0.00±0.00	14.28±0.65	18.29±0.50	21.40±0.56
24	*Parthenium hysterophorus*	0.00±0.00	0.00±0.00	26.59±0.54	32.32±0.63	37.16±0.93
	Lantana camara	0.00±0.00	0.00±0.00	36.18±0.59	31.22±0.63	27.22±0.56
	Hyptis suaveolens	0.00±0.00	0.00±0.00	24.04±0.80	22.46±0.46	30.35±0.51
	Sida acuta	0.00±0.00	0.00±0.00	16.68±0.47	21.19±0.62	28.48±0.65
36	*Parthenium hysterophorus*	0.00±0.00	0.00±0.00	67.24±0.57	69.56±0.59	74.34±0.58
	Lantana camara	0.00±0.00	0.00±0.00	70.22±0.63	68.37±0.47	64.50±0.60
	Hyptis suaveolens	0.00±0.00	0.00±0.00	32.34±0.46	34.46±0.62	46.39±0.57
	Sida acuta	0.00±0.00	0.00±0.00	31.12±0.63	33.53±0.54	43.26±0.58
48	*Parthenium hysterophorus*	0.00±0.00	0.00±0.00	73.57±±0.44	78.24±0.47	82.38±0.64
	Lantana camara	0.00±0.00	0.00±0.00	73.54±0.35	70.11±0.67	66.62±0.55
	Hyptis suaveolens	0.00±0.00	0.00±0.00	38.76±0.62	46.47±0.60	58.69±0.63
	Sida acuta	0.00±0.00	0.00±0.00	36.61±0.71	42.25±0.56	52.50±0.50
SEm±		0	0	0.53	0.64	0.45
$LSD_{5\%}$		0	0	1.26	1.42	1.38

Values are Means ± SEM of three observations; Control a- Unmetabolised growth medium; Control b- Sterilized Distilled Water; Amount of Toxin employed=5 ml/leaf; RH-85% PDR- 0-0.99= slight curling & wilting; 1-1.99= 1-20%LAD; 2-2.99=21-40% LAD; 3-3.99= 41-60% LAD; 4-4.99 = 61-80%LAD; 5 = 81 100%LAD (leaf area damage)

Table 4: Reduction in protein content due to TLC purified phytotoxins in test weeds (Phytotoxin II)

Incubation hours (hpt)	Weeds	Control a	Control b	% Reduction in protein
12	*Parthenium hysterophorus*	0.00±0.00	0.00±0.00	63.39 ±0.69
	Lantana camara	0.00±0.00	0.00±0.00	60.69±0.82
	Hyptis suaveolens	0.00±0.00	0.00±0.00	56.73±0.85
	Sida acuta	0.00±0.00	0.00±0.00	49.66±0.84
24	*Parthenium hysterophorus*	0.00±0.00	0.00±0.00	70.49 ± 0.57
	Lantana camara	0.00±0.00	0.00±0.00	67.61± 0.76
	Hyptis suaveolens	0.00±0.00	0.00±0.00	61.49±0.65
	Sida acuta	0.00±0.00	0.00±0.00	58.65±0.71
36	*Parthenium hysterophorus*	0.00±0.00	0.00±0.00	78.64±0.64
	Lantana camara	0.00±0.00	0.00±0.00	73.43±0.69
	Hyptis suaveolens	0.00±0.00	0.00±0.00	70.34±0.76
	Sida acuta	0.00±0.00	0.00±0.00	64.33±0.59
48	*Parthenium hysterophorus*	0.00±0.00	0.00±0.00	81.36±0.68
	Lantana camara	0.00±0.00	0.00±0.00	79.15±0.61
	Hyptis suaveolens	0.00±0.00	0.00±0.00	73.44±0.66
	Sida acuta	0.00±0.00	0.00±0.00	72.22±0.65
SEm±		0	0	0.56
$LSD_{5\%}$		0	0	1.63

Values are Means ± SEM of three observations; Control a- Unmetabolised growth medium; Control b- Sterilized Distilled Water; Amount of Toxin employed=5 ml/leaf RH-85% PDR- 0-0.99= slight curling & wilting; 1-1.99= 1-20%LAD; 2-2.99=21-40% LAD; 3-3.99= 41-60% LAD; 4-4.99 = 61-80%LAD; 5 = 81 100%LAD (leaf area damage)

Table 5: Electrolyte leakage studies with Phytotoxin I.

Incubation hours (hpt)	Weeds	Control	Conductivity Values µ Mhos (26˚C)
0	**Parthenium hysterophorus**	0.00±0.00	0.00±0.00
	Lantana camara	0.00±0.00	0.00±0.00
	Hyptis suaveolens	0.00±0.00	0.00±0.00
	Sida acuta	0.00±0.00	0.00±0.00
1	**Parthenium hysterophorus**	0.00±0.00	6.55±0.55
	Lantana camara	0.00±0.00	6.12±0.74
	Hyptis suaveolens	0.00±0.00	3.31±0.64
	Sida acuta	0.00±0.00	2.93±0.61
2	**Parthenium hysterophorus**	0.00±0.00	10.14±0.56

	Lantana camara	0.00±0.00	8.49±0.59
	Hyptis suaveolens	0.00±0.00	6.71±0.56
	Sida acuta	0.00±0.00	4.48±0.66
3	**Parthenium hysterophorus**	0.00±0.00	14.38±0.39
	Lantana camara	0.00±0.00	12.46±0.53
	Hyptis suaveolens	0.00±0.00	9.37±0.60
	Sida acuta	0.00±0.00	7.65±0.61
SEm±		0	0.50
$LSD_{5\%}$		0	1.45

Values are Means ± SEM of three observations; Control - Sterilized Double Distilled Water; Amount of Toxin employed=1 ml/50 mg of weed leaves

Table 6: Electrolyte leakage studies with Phytotoxin II

Incubation hours (hpt)	Weeds	Control	Conductivity Values µ Mhos (26˚C)
0	*Parthenium hysterophorus*	0.00±0.00	0.00±0.00
	Lantana camara	0.00±0.00	0.00±0.00
	Hyptis suaveolens	0.00±0.00	0.00±0.00
	Sida acuta	0.00±0.00	0.00±0.00
1	*Parthenium hysterophorus*	0.00±0.00	5.43±0.44
	Lantana camara	0.00±0.00	4.35±0.58
	Hyptis suaveolens	0.00±0.00	2.45±0.30
	Sida acuta	0.00±0.00	2.19±0.30
2	*Parthenium hysterophorus*	0.00±0.00	6.86±0.57
	Lantana camara	0.00±0.00	7.69±0.63
	Hyptis suaveolens	0.00±0.00	5.57±0.60
	Sida acuta	0.00±0.00	3.45±0.62
3	*Parthenium hysterophorus*	0.00±0.00	13.61±0.70
	Lantana camara	0.00±0.00	11.45±0.40
	Hyptis suaveolens	0.00±0.00	8.77±0.58
	Sida acuta	0.00±0.00	6.55±0.58
SEm±		0	0.47
$LSD_{5\%}$		0	1.37

Values are Means ± SEM of three observations; Control - Sterilized Double Distilled Water; Amount of Toxin employed=1 ml/50 mg of weed leaves