INT. J. BIOL. BIOTECH., **17 (2): 339-345, 2020.**

COMPARATIVE STUDY ON THE NEMATICIDAL ACTIVITY OF FRUIT AND SEEDS FRACTIONS OF *CITRULLUS COLOCYNTHIS* L. SCHRAD. AGAINST *MELOIDOGYNE INCOGNITA*

Sonam Khatri[1*], Shahina Fayyaz[1], Shaheen Faizi[2], Shahnaz Dawar[1], Erum Y. Iqbal[1] and Salma Javed[1]

[1]*National Nematological Research Centre, University of Karachi, Karachi 75270, Pakistan*
[2]*HEJ Research Institute of Chemistry, International Center for Chemical and Biological Sciences, University of Karachi, Karachi 75270, Pakistan*
*Corresponding author: khatrisonam9@gmail.com

ABSTRACT

The current study was carried out to evaluate the nematicidal potential of different extracts of seeds, ripe fruit without seeds, unripe green fruit and powdered fruit of *Citrullus colocynthis* against second stage juveniles of root-knot nematodes, *Meloidogyne incognita*. The activities of extracts were examined *in vitro* and was compared with the positive control (Furadan). This study gave an overview of nematicidal potential of *C. colocynthis* and showed great activity up to 99% mortality of juveniles of *M. incognita* in some extracts. Ethyl acetate (EA) and Methanolic extract (Me) of *Citrulllus* seed (CS) have shown 99.33 and 98.66% mortality, respectively, 92.66% mortality was obtained from Me extracts of green fruit (CG) whereas Petroleum ether (PE) and (EA) extracts of powdered fruits were found 98.00 and 94.66% effective against *M. incognita* after 72 hrs of exposure. This study concludes the use of this fruit as a potential source of bio-pesticide.

Key Word: *Citrullus colocynthis*; *Meloidogyne incognita*; Nematicidal Activity; Biopesticides; Fruit and seeds fractions.

INTRODUCTION

Plant parasitic nematodes are among the most destructive and prevalent pests of agricultural crops which are responsible for major economic losses of an estimated USD 118 billion in a single 5 years on agriculture and destroying at least 12.3% of global food production annually (Ahmed *et al.*, 2015; Hassan *et al.*, 2013).

Among them, root-knot nematodes, *Meloidogyne* species are most menacing and tough to manage due to their inhabitation in the soil and short and productive life cycle (Renco, 2013). Management of phytonematodes in field crops has up to now been reliant on the use of nematicides which are being gradually phased out following the awareness of the influence of these nematicides on the environment (Hague and Gowen, 1987; Akhtar and Malik, 2003). Alternative measures are now considered due to the unreliable consequences of nematicides currently in practice, chiefly due to enhanced biodegradation and aggravation of various diseases like asthma, diabetes, leukemia and reproductive, gastric, cardiovascular and nervous disorders etc. (Qin *et al.*, 2004; Giannakou *et al.*, 2005). Persistent use of pesticides not only affects the human health but also have negative impact on biodiversity and the food chain (Jayaraj *et al.*, 2016). All these factors create a crucial requisite for alternative nematode control measures that is eco-friendly substitutes to manage plant-parasitic nematode populations.

Plants have been the treasure of bioactive compounds which play crucial role in their own defense mechanism that are used as the source of natural pesticides for centuries. Therefore, plant extract may be better substitutes of synthetic ones. Natural plant products are at the present research focus because of their ability to produce environmentally less harmful but efficacious chemical substances (Chitwood, 2002). Number of studies showed the nematicidal bioactivities in extracts of different plant families against phytonematodes (Faizi *et al.*, 2011; Leonetti *et al.*, 2011; Caboni *et al.*, 2012).

Citrullus colocynthis (L.) Schrad, a member of the Cucurbitaceae family, grown as a wild perennial in desert areas of the world including Pakistan with noteworthy potential for medicinal and nutraceutical applications along with promising pesticidal properties tested against various economical pests (Asyaz *et al.*, 2010; Sawaya *et al.*, 1983; Arivoli *et al.*, 2012; Hamid *et al.*, 2016; Khalid, 2015; Akpotu *et al.*, 2017). Only few studies reported nematicidal properties of *Citrullus colocynthis* and showed the promising effects against different species of nematodes (Mohammad *et al.*, 1981; Muniasamy *et al.*, 2010; Rizvi and Shahina, 2014).

In the current study, we used the ripe fruit, green unripe fruits, seeds and powdered fruit of *C. colocynthis* for the evaluation of their nematicidal potential against root-knot nematodes.

MATERIALS AND METHODS

Plant material

The fresh ripe and unripe fruits of *Citrullus colocynthis* (L.) Schrad. were purchased in the month of March, 2016 from local market of Karachi, Pakistan and identified by plant taxonomist Dr. Anjum Parveen, Centre of Plant Conservation, University of Karachi. A voucher specimen (PC-1899) was deposited in the herbarium of the same department.

Chemicals

All the chemicals and solvents used were of analytical grade. Dimethyl sulfoxide was purchased from Merck Germany.

Extraction:

Extraction of seeds

Seeds (30 g) incrusted from the pulp of the ripe fruits were ground in basal mortar and were extracted successively with petroleum ether (PE), dichloromethane (DCM), ethyl acetate (EA), acetone (Ac) and methanol (Me). All the extracts were evaporated at room temperature furnishing respective residues, CS-PE, CS-DCM, CS-EA, CS-Ac and CS-Me as shown in Fig.**1**.
(CS= *Citrullus* seeds).

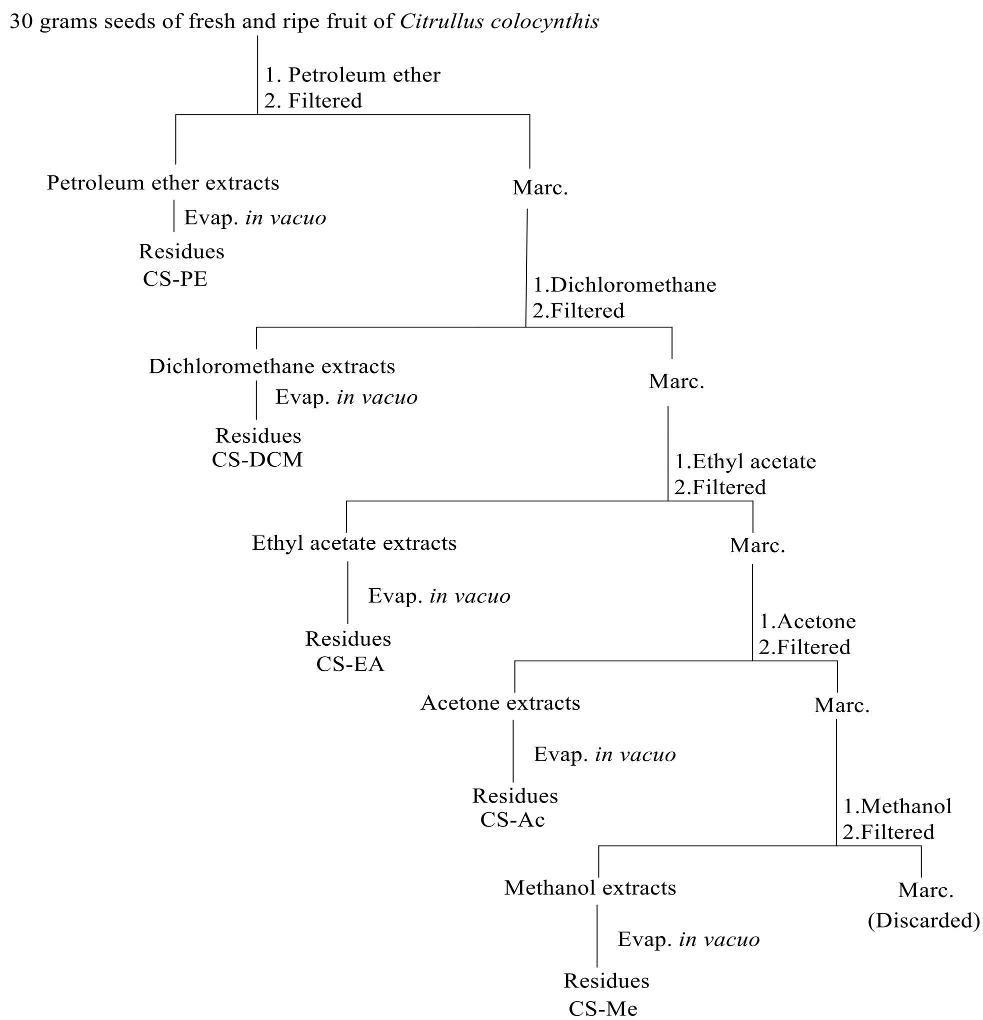

Fig.1. Successive extraction of seeds of *Citrullus colocynthis* at room temperature.

Extraction of green, unripe fruit, ripe fruits without seeds and powdered fruit

Similar extraction protocol followed with green, unripe fruit (134 g) and fresh ripe, undried fruits without seeds (410 g) which were extracted successively with PE followed by DCM, EA, Ac and Me. Powdered fruit (343 g) was also extracted successively but DCM was skipped and extraction was done with PE, EA, Ac and Me.

Root-knot culture

Pure culture of plants *Solanum melongena* were maintained in micro plot of a screen house in National Nematological Research Center, University of Karachi, Karachi, Pakistan. Egg masses were extracted from the roots of infected plant and were incubated at 28 ˚C for 3 days in tap water.

Nematicidal activity

Experiments were conducted in *in vitro* conditions. The nematicidal potential of extracts of seeds, ripe fruits without seeds, green fruits and powdered fruit of *C. colocynthis* was evaluated against freshly hatched J_2 stage of root-knot nematode. Egg-masses of root-knot nematodes from the prepared culture at National Nematological Research Centre, University of Karachi, were picked out and placed for hatching in room temperature. After 24 h, 100 freshly hatched second-stage juveniles were placed separately in different concentrations (1, 0.5 and 0.125%) of the extracts. After different time durations (24, 48 and 72 h of intervals), the movement of the nematodes was tested by touching them with the needle, the larval mortality was observed and counted under a stereoscopic microscope. After 72 h of exposure, the nematodes which had shown mortality were transferred to a separate glass cavity block having distilled H_2O. Next observation was taken after 24 h for the confirmation of their mortality (Samina *et al*., 2019). Conventional nematicide, “Furadan” was kept as standard applied in parallel for comparison and 1% aq. DMSO was used as a negative control. The experiment was repeated thrice.

Statistical analysis

LD_{50} was calculated through probit values with the following formula:

$LD_{50} = \log^{-1} \{xm – i(\Sigma p-0.50\}$
$X=\log^{-1} (\log LD_{50} + 1.96 \times i \sqrt{\Sigma p(1-p/n)}$
Xm = dosage logarithm for the maximal dosage; i = the difference of dosage logarithm between two nearest groups; p = death rate of nematodes in each group; X = 95% confidence intervals; n = number of the nematodes in each group.

RESULTS AND DISCUSSION

Botanical nematicides are the source of alternative bio-rational and eco-safe products to toxic synthetic nematicides. Very little work has been done to consider the potential of *C. colocynthis* to manage phytonematodes. Sensitivity of nematicides varies among different species of plant-parasitic nematodes. In general, J2s of *Meloidogyne* spp. shows sensitivity to plant-derived nematicides (Oka *et al.*, 2001).

In the present study the extracts of seeds, ripe fruits, green fruits and powder of *Citrullus colocynthis* in different solvents were evaluated on second stage of *M. incognita* under laboratory studies. Interpretation of results of the present study clearly demonstrates the variation in effectiveness of concentrations and exposure time of extracts on juvenile mortality. Different extracts of different parts showed great variation in their nematicidal potential. Only few extracts showed poor response against nematodes while most of the extracts showed great activity.

The EA and Me extract of seeds showed greatest activity of 99.33 and 98.66% at 1% concentration after 72 h. The activity of EA extract slightly declined with decreasing concentration of the extract but still killed 86.66 and 76% juveniles while Me extract killed 80% and 60.66 at 0.5 and 0.125% concentrations, respectively after 72 h. PE, DCM and Ac extracts of seeds showed moderate activity at 1% concentration but at lower concentration they showed poor response against juveniles (Table 1). The seed extract was oily and viscous due to its composition which is mainly a mixture of fatty acids (Nehdi *et al*., 2013); and seed oil of *C. colocynthis* was concluded as potential biocide against phytonemtodes which supports our results (Tarraf *et al.*, 2019). Along with promising nematicidal activity, seed extracts of *C. colocynhis* also possess insecticidal properties (Nzelu and Okonkwo, 2016; Akpotu *et al.*, 2015; Dimetry *et al.*, 2015; Dimetry *et al.*, 2007).

The Me extract of green fruit showed highest mortality of 92.66% followed by DCM and EA extracts which showed good activity of 85.33 % and 84.33%, respectively at 1% after 72 h and decreases with the concentration of extract. DCM and Me extract showed moderate activity even after 24 and 48 h at 1 and 0.5% concentrations but EA extract showed poor response after 24 h at all concentration while after 48 h it showed moderate activity which clearly demonstrates that the exposure time is directly proportional to the mortality of nematodes. PE extracts showed lower activity at all concentrations even after 72 h. While Ac extract displayed worst activity against nematodes as at 0.125% it does not killed a single nematode (Table 1).

Table 1. Mortality percentage of *Meloidogyne incognita* in various extracts of *Citrullus colocynthis* at different time intervals.

S.NO	Sample code	Conc. (%)	After 24 h Mean ± SD	After 48 h Mean ± SD	After 72 h Mean ± SD	LD_{50} After 72 h
CS: Successive extraction of seeds of ripe summer fruit						
1	CS-PE	1	20.00 ± 0.81	34.66 ± 2.05	46.33 ± 1.24	1.053
		0.5	8.00 ± 1.63	15.33 ± 0.81	25.66 ± 1.24	
		0.125	0.00 ± 0.00	6.33 ± 0.47	18.33 ± 1.69	
2	CS-DC	1	10.00 ± 1.63	24.66 ± 0.81	53.66 ± 4.10	0.910
		0.5	10.33 ± 1.24	16.33 ± 1.24	41.00 ± 2.94	
		0.125	0.00 ± 0.00	11.33 ± 2.62	33.33 ± 2.05	
3	CS-EA	1	55.00 ± 1.69	86.33 ± 1.24	99.33 ± 0.94	0.102
		0.5	44.66 ± 2.49	67.33 ± 3.77	86.66 ± 1.69	
		0.125	31.00 ± 2.86	62.33 ± 2.05	76.00 ± 0.81	
4	CS-Ac	1	19.33 ± 0.47	33.00 ± 0.81	60.33 ± 1.24	0.725
		0.5	12.33 ± 0.47	29.33 ± 0.94	42.33 ± 2.05	
		0.125	03.33 ± 0.47	12.00 ± 0.81	22.00 ± 2.94	
5	CS-Me	1	62.00 ± 1.63	85.00 ± 0.81	98.66 ± 0.47	0.098
		0.5	47.66 ± 1.24	65.33 ± 1.94	80.00 ± 1.63	
		0.125	32.66 ± 0.47	42.00 ± 1.41	60.66 ± 2.05	
CG: Successive extraction of green summer fruit						
6	CG-PE	1	7.00 ± 0.8	20.33 ± 0.47	48.00 ± 0.81	1.226
		0.5	4.66 ± 0.47	14.66 ± 0.47	34.66 ± 1.24	
		0.125	0.00 ± 0.00	6.66 ± 1.69	20.33 ± 1.24	
7	CG-DC	1	44.33 ± 0.94	57.00 ± 2.16	85.33 ± 2.86	0.130
		0.5	31.66 ± 0.47	51.66 ± 1.69	65.66 ± 3.29	
		0.125	18.00 ± 2.16	26.66 ± 1.69	51.33 ± 3.39	
8	CG-EA	1	27.00 ± 2.16	63.33 ± 2.35	84.33 ± 3.29	0.090
		0.5	24.00 ± 0.81	51.00 ± 0.81	76.00 ± 2.16	
		0.125	15.66 ± 1.24	36.33 ± 2.51	62.33 ± 2.62	
9	CG-Ac	1	0.00 ± 0.00	08.00 ± 2.16	22.33 ± 3.09	2.434
		0.5	0.00 ± 0.00	05.00 ± 0.81	14.33 ± 0.47	
		0.125	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	
10	CG-Me	1	45.33 ± 1.24	72.33 ± 0.47	92.66 ± 1.69	0.822
		0.5	29.33 ± 0.94	51.00 ± 0.81	70.33 ± 0.47	
		0.125	17.00 ± 1.41	35.33 ± 1.24	59.33 ± 1.24	
CR: Successive extraction ripe summer fruit without seeds						
11	CR-PE	1	10.00 ± 0.81	17.33 ± 1.69	32.33 ± 2.05	1.403
		0.5	6.33 ± 1.24	15.33 ± 1.24	27.66 ± 1.24	
		0.125	0.00 ± 0.00	10.00 ± 0.81	19.66 ± 0.47	
12	CR-DC	1	24.00 ± 1.63	53.66 ± 1.69	76.00 ± 2.44	0.628
		0.5	9.66 ± 0.47	35.00 ± 5.09	53.33 ± 2.49	
		0.125	6.33 ± 0.94	22.33 ± 2.05	44.66 ± 3.68	
13	CR-EA	1	24.66 ± 1.24	46.33 ± 1.24	65.66 ± 2.49	0.482
		0.5	13.00 ± 1.41	24.66 ± 2.05	52.66 ± 2.49	
		0.125	9.66 ± 0.47	19.66 ± 1.24	36.00 ± 1.63	

14	CR-Ac	1	30.33 ± 1.24	50.66 ± 0.94	70.66 ± 0.47	
		0.5	20.00 ± 1.41	40.33 ± 0.94	53.33 ± 1.24	
		0.125	0.00 ± 0.00	20.66 ± 1.69	38.00 ± 1.63	0.561
15	CR-Me	1	23.00 ± 0.81	53.33 ± 1.24	88.00 ± 0.41	
		0.5	12.66 ± 1.88	31.33 ± 0.94	67.35 ± 2.35	
		0.125	02.33 ± 1.24	15.00 ± 1.41	40.66 ± 2.86	0.478
TD: Successive extraction of powdered fruit.						
16	TD-PE	1	50.33 ± 3.39	67.33 ± 5.55	98.00 ± 2.16	0.062
		0.5	52.00 ± 4.96	74.00 ± 2.16	90.66 ± 3.09	
		0.125	49.33 ± 1.69	67.00 ± 2.16	92.33 ± 2.05	
17	TD-EA	1	49.33 ± 3.29	78.33 ± 4.78	94.66 ± 4.18	0.084
		0.5	45.33 ± 0.47	73.33 ± 4.49	86.00 ± 2.16	
		0.125	30.00 ± 0.81	62.33 ± 2.05	75.00 ± 1.41	
18	TD-Ac	1	9.66 ± 0.57	22.33 ± 1.52	31.66 ± 1.15	
		0.5	0.00 ± 0.00	7.00 ± 2.00	20.66 ± 2.51	
		0.125	0.00 ± 0.00	0.00 ± 0.00	10.66 ± 1.15	1.885
19	TD-Me	1	14.00 ± 2.00	36.33 ± 0.57	63.00 ± 2.00	
		0.5	10.66 ± 0.57	20.33 ± 1.52	50.00 ± 1.73	
		0.125	5.33 ± 1.52	18.66 ± 1.52	40.33 ± 0.57	0.504
Positive and negative controls						
20	Positive control (Furadan)	1	100.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	
		0.5	98.33 ± 0.47	1.66 ± 0.47	0.00 ± 0.00	
		0.125	97.66 ± 0.94	2.33 ± 0.94	0.00 ± 0.00	0.056
21	Negative control ($DMSO_4$)	1	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	
		0.5	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	
		0.125	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00

Likewise green fruit, Me extract of ripe fruit was also recognized as most nematicidal among other extracts and showed 88% mortality followed by DCM extract which showed 76% mortality at 1% concentration after 72 h. EA and Ac extracts showed good to moderate activity at high concentration after 72 hours while PE extract showed poor activity over all. Nematicidal efficiency of both ripe and unripe fruit seems to be similar, no such variation in nematicidal efficiency of any extract was observed. Other than nematodes, in various studies, pesticidal activity of *C. colocynthis* fruit extracts have been evaluated against many pests like cotton leaf worm, *Spodoptera littoralis*; locust, *Chrotogonus Trachypterus*; red spider mite, *Teteanychus urticae;* rice weevil, *Sitophilus oryzea;* maize weevil, *Sitophilus zeamais* and cowpea aphid, *Aphis craccivora*; *Bactrocera zonata* and discovered excellent results (Rawi *et al.*, 2011; Mollashahi *et al.*, 2017; Jeon and Lee, 2014; Torkey *et al.*, 2009; Rehman *et al.*, 2009).

The nematicidal activity of powdered fruit of *C. colocynthis* is slightly different, as the PE extracts of seeds, ripe and unripe fruits showed least activity against juveniles but PE extract of powder showed highest activity of 98% at 1% concentration which slightly declined with the decreasing concentration showing 90.66 and 92.33% after 72 h at 0.5 and 0.125% concentrations, respectively. EA extract also showed great activity at all doses after 72 hours. But unlike fruits and seeds, Me and Ac extracts showed least activity against nematodes. Nematicidal activity of powdered fruit is not reported yet but according to some studies, ethanol extract of *C. colocynthis* fruit powder found to be effective against *Tribolium castaneum* and in another study, ethyl acetate extract of powdered fruit was most effective after ethanol extract to control the cotton ballworm, *Helicoverpa armigera* (Gulzar *et al.*, 2017; Nadeem *et al.*, 2012).

The results suggest that EA and Me extracts of seeds, DCM, EA and Me extracts of unripe fruits, all the extracts except PE extracts of ripe fruits and PE and DCM extracts of powdered fruit have considerable potential at different concentrations as bio-pesticide against phytoparasitic nematodes. Practically, the use of this plant is easier as it is a wild plant which grows easily in desert and does not require much water for nourishment, so it could be easily available resource to the poor farmers. This is the first study in which nematicidal potential of powdered fruits extracts of *C. colocynthis* were evaluated and found to be efficient. From this study, it can be concluded that the use of botanical means may serve as an alternative to the chemicals in order to bring sustainability to agriculture. However, further studies on the chemical characterization of the extracts must be done to identify the bioactive compound responsible for the nematicidal potential and new ways must be assessed to introduce a new bio-rational product for the use in integrated pest management.

Acknowledgements

The present research is the part of Sonam Khatri's dissertation entitled "The effect of *Citrullus colocynthis* extracts against phytonematodes", National Nematological Research Centre, University of Karachi, 2020.

REFERENCES

Ahmed, M., M. Sapp, T. Prior, G. Karssen and M. Back (2015). Nematode taxonomy: from morphology to metabarcoding. *Soil Discussions*, 2: 1175-1220.

Akhtar, M. and A. Malik (2003). Roles of organic soil amendments and soil organisms in the biological control of plant-parasitic nematodes: a review. *Bioresource Technology*, 74: 35–47.

Akpotu, O. J., S. J. Oniye, D. Abolude and A. Yusuf (2015). Comparative study of the toxicity of oils from seeds of *Citrullus colocynthis* and *Citrullus vulgaris* on larvae of *Dermestes Maculatus*. *Journal of Aquaculture Engineering and Fisher Research*, 3(1): 6-12.

Arivoli, S., K.J. Ravindran and T. Samuel (2012). Larvicidal efficacy of plant extracts against the malarial vector *Anopheles stephensi Liston* (Diptera: Culicidae). *World Journal of Medical Sciences*. 7(2):77–80.

Asyaz, S., F.U. Khan, I. Hussain, M.A. Khan and I.U. Khan (2010). Evaluation of chemical analysis profile of *Citrullus colocynthis* growing in Southern area of Khyber Pukhtunkhwa, Pakistan. *Journal of World Applied Sciences*, 10: 402-405.

Caboni, P., N.G. Ntalli, N. Aissani,I. Cavoski and A. Angioni (2012). Nematicidal activity of (E, E)-2,4-decadienal and (E)-2decenal from *Ailanthus altissima* against *Meloidogyne javanica*. *Journal of Agriculture and Food Chemistry*, 60: 1146–115.

Chitwood, D.J. (2002). Phytochemical based strategies for nematode control. *Annual Review of Phytopathology* 40: 221–249.

Dimetry, N.Z., S. El-Gengaihi and A.E.M. Abd El-Salam (2007). Protection of stored cowpea from *Callosobruchus maculatus* (F.). *Herba Pol*, 53(1): 71-84.

Dimetry, N.Z., S. El-Gengaihi, M. Hafez and M.H. Abbass (2015). Pesticidal activity of certain plant extracts and their isolates against the cowpea beetle *Callosobruchus maculatus* (F.) (Coleoptera: Chrysomelidae: Bruchinae). *Herba polonica*. 61(3): 77-92.

Faizi, S., S. Fayyaz, S. Bano, E.Y. Iqbal, L. Lubna, H. Siddiqi and A. Naz (2011). Isolation of nematicidal compounds from *Tagetes patula* L. yellow flowers: structure-activity relationship studies against cyst nematode *Heterodera zeae* infective stage larvae. *Journal of Agriculture and Food Chemistry*, 9: 9080–9093.

Giannakou, I.O., D.G. Karpouzas, I. Anastasiades, N.G. Tsiropoulos and A. Georgiadou (2005). Factors affecting the efficacy of non-fumigant nematicides for controlling root-knot nematodes. *Pest Management Sciences*, 61: 961–972.

Gulzar, A., A. Maqsood, M. Ahmed, M. Tariq, M. Ali and R. Qureshi (2017). Toxicity, antifeedant and sub-lethal effects of *Citrullus colocynthis* extracts on cotton bollworm, *Helicoverpa armigera* (Lepidoptera: Noctuidae). *Pakistan Journal of Zoology*, 49(6): 2019-2026.

Hague, N. and S. Gowen (1987). Chemical control of nematodes. *Principle and Practice of Nematodes Control in crops*, 131-178.

Hamid, N.S., M.A. Kahil and N.A. Ibrahim (2016). Larvicidal activity of ethanol extract of *Citrullus colocynthis* seed and fruit pulp against *Anopheles arabiensis* and *Culex quinquefasciatus*. *Journal of Medicinal Plants Studies*, 4(6): 252-255.

Hassan, M. A., T. H. Pham, H. Shi and J. Zheng (2013). Nematodes threats to global food security. *Acta Agriculturae Scandinavica, Section B–Soil & Plant Science*, 63(5): 420-425.

Jayaraj, R., P. Megha and P. Sreedev (2016). Organochlorine pesticides, their toxic effects on living organisms and their fate in the environment. *Interdisciplinary toxicology*, 9(3-4): 90-100.

Jeon, J.H., and H.S. Lee (2014). Biofunctional constituent isolated from *Citrullus colocynthis* fruits and structure–activity relationships of its analogues show acaricidal and insecticidal efficacy. J*ournal of Agriculture Food and Chemistry*, 62: 8663–8667.

Khalid, A.A. (2015). Aphidicidal activity of different aqueous extracts of bitter apple *Citrullus colocynthis* (L.) against the bird cherry-oat aphid, *Rhopalosiphum padi* (L.) (Homoptera: Aphididae) under laboratory conditions. *The Journal of Animal & Plant Sciences*, 25 (2): 456-462.

Leonetti, P., T. D'Addabbo, P. Avato and A. Tava (2011). Control of root-knot nematodes with biomasses from alfalfa (*Medicago sativa* L.) and their bioactive saponins. *Acta Hortic*, 914: 225–228.

Mohammad, H.Y., S.I. Husain and A.J. Al-Zarari (1981). Effect of plant extracts of some poisonous plants of Iraq on mortality of citrus nematode, *Tylenchulus semipenetrans* Cobb. *Acta Botanica Indica*, 9: 198-200.

Mollashahi, H., A. Mirshekari, M.Ghorbani and A. Tarrah (2017). Insecticidal effect of the fruit extract bitter melon (*Citrullus colocynthis*) on locust *Chrotogonus trachypterus* (Orth: Pyrgomorphidae). *Bioscience Biotechnology Research Asia*, 14(4): 1285-9.

Muniasamy, S., M. Pavaraj and M. K. Rajan (2010). Efficacy of the fruit extract of *Citrullus colocynthis* (L.) on the root-knot nematode *Meloidogyne incognita* infecting *Vigna ungiculata* (L.). *Journal of Biopesticides*, 3(1): 309-312.

Nadeem, M., J. Iqbal, M.K. Khattak 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(5): 1325–1331.

Nehdi, I. A., H. Sbihi, C. P. Tan and S. I. Al-Resayes (2013). Evaluation and characterisation of *Citrullus colocynthis* (L.) Schrad seed oil: Comparison with *Helianthus annuus* (sunflower) seed oil. *Food Chemistry*, 136(2): 348-353.

Nzelu, C. O. and N. J. Okonkwo (2016). Evaluation of melon seed oil *Citrullus colocynthis* (L.) Schrad, for the protection of cowpea *Vigna unguiculata* seeds against *Callosobruchus maculatus* (Fabricius) (Coleoptera: Bruchidae). *Evaluation*, 3(8): 1-10.

Oka, Y. (2001). Nematicidal activity of essential oil components against the root-knot nematode *Meloidogyne javanica*. *Nematology*, 3: 159–64.

Qin S.J., J.Y. Can, W.P. Liu and J.O. Becker (2004). Degradation and adsorption of fosthiazate in soil. *Journal of Agriculture and Food Chemistry*, 52: 6239–6242.

Rawi, S. M., F. A. Bakry and M. A. Al-Hazmi (2011). Biochemical and histopathological effects of crude extracts on *Spodoptera littoralis* larvae. Journal of *Evolutionary Biology Research*, 3(5): 67-78.

Rehman, J., G. Jilani, M.A. Khan, R. Masih and S. Kanvil (2009). Repellent and oviposition deterrent effects of indigenous plant extracts to peach fruit fly, *Bactrocera zonata saunders* (Diptera: Tephritidae). *Pakistan Journal of Zoology*. 41(2): 101–8.

Renco, M. (2013). Organic amendments of soil as useful tools of plant parasitic nematodes control. *Helminthologia*, 50(1): 3-14.

Rizvi, T.S. and F. Shahina (2014). Nematicidal activity of *Citrullus colocynthis* extracts against root-knot nematodes. *Pakistan Journal of Nematology*, 32: 101–112.

Samina, B., F. Shaheen, Lubna, F. Shahina and Y. I. Erum (2019). Isolation of Ceramides from *Tagetes patula* L. yellow flowers and nematicidal activity of the fractions and pure compounds against cyst nematode, *Heterodera zeae*. 16: 16. https://doi.org/10.1002/cbdv.201900092

Sawaya, W.N., N.J. Daghir and P. Khan (1983). Chemical characterization and edibility of the oil extracted from *Citrullus colocynthis* seeds. *Journal of Food Science*, 48: 104-106.

Tarraf, W., S. Laquale, G. De Mastro and T. D'Addabbo (2019). The potential of *Citrullus colocynthis* oil as a biocide against phytoparasitic nematodes. *Crop Protection*, 104843.

Torkey, H.M., H.M. Abou-Yousef, A.Z. Abdel Azeiz and E.A.F. Hoda (2009). Insecticidal effect of Cucurbitacin E Glycoside isolated from *Citrullus colocynthis* against *Aphis craccivora*. *Australian Journal of Basic and Applied Science*, 3(4): 4060-4066.

(*Accepted for publication March 2020*)