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IDENTIFICATION OF PHENOLICS IN MANGO LEAVES EXTRACT AND THEIR ALLELOPATHIC EFFECT ON CANARY GRASS AND WHEAT

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Abstract

Phytotoxic ability of aqueous extracts of new and old mango leaves at different concentrations i.e., 2.5, 5.0, 7.5 and 10% against canary grass and wheat were tested *In vitro* and *In vivo*. Results revealed that all the extracts significantly inhibited the germination and growth of canary grass *In vitro* as well as *In vivo*. Maximum inhibition i.e. more than 80% to all the parameters of canary grass was observed by 10% extract *In vitro*. In pot experiment 66% reduction, in the dry weight of canary grass was observed when the old leaves water extract was applied before emergence. All the treatments showed non significant results about the germination of wheat *In vivo*. Old mango leaves extract was found better as compared to new ones, because it moderately enhanced the wheat germination and growth, while new mango leaves induced some reduction regarding the shoot length and grain weight of wheat. Total phenolic contents were higher in new mango leaves as compared to old ones. 4-hydroxybenzaldehyde, *m*-coumaric, *p*-coumaric, 4-hydroxy benzoic, vanillic, caffeic, gallic and protocatechuic acids were phenolic compounds identified through Mass Spectrometry and High Performance Liquid Chromatography analyses of mango leaves. It has been concluded that old mango leaves extract could be used as a herbicide to suppress canary grass and to enhance wheat growth.

Introduction

The weed spectrum of wheat (*Triticum aestivum* L.) in Pakistan consists of a number of weeds, among which little seed canary grass locally known as dumbi citi (*Phalaris minor* Retz.) is a problematic annual grass weed (Om *et al*., 2004). Due to crop mimicry, it is difficult to differentiate this weed from wheat at the seedling stage as their seedlings are identical to wheat seedlings in morphology (Hassan *et al*., 2005). Losses caused by weeds can be as high as 24% of the yield compared with 16.4 and 11.2% for disease and pests, respectively (Oerke & Steiner, 1996; Memon *et al.,* 2013). The increasing dependence on chemicals for weed control poses a serious threat to human health and environment due to their persistent nature (Macias *et al*., 1999; Ibrahim *et al.,* 2013). Little seed canary grass has also developed resistance to the commonly used herbicide isoproturon (Paveglio & Kilbride, 1996). Therefore, integrated weed management approaches are emphasized. The use of natural compounds from different allelopathic crops, against weeds besides enhancing production potential of economically important crop can provide an alternative or a complementary tactics for sustainable integrated weed management (Anjum & Bajwa, 2007).

Allelopathy is a natural and environment friendly technique which may prove to be a tool for weed management and thereby increase crop yields (Han *et al.,* 2013). So, the term allelopathy is commonly denotes the interaction in which one plant could cause suffering to another plant. The main principle in allelopathy arises from the fact that plants produce thousands of chemicals; and many of these chemicals are released by leaching, exudation, or decomposition processes. Subsequently, some of these compounds which are known as allelochemicals alter the growth or physiological functions of receiving species. The most commonly found allelochemicals are cinnamic and benzoic acids, flavonoids, and various terpenes (Singh *et al*., 2003). These compounds are known to be phytotoxic (Einhellig, 2002). Hence, plants are a vast source of naturally occurring selective herbicides, which may be environment friendly. It could be extracted from flower, leaves, stem and roots. These allelopathic extracts could be used to control the growth of weeds (Chon & Kim, 2002; 2004; Chon *et al*., 2003; Singh *et al*., 2003; El-Rokiek *et al*., 2006).

It is worthy to mention that mango leaves either in aqueous extract or soil applied powder induce significant reduction in purple nutsedge growth (El-Rokiek *et al*., 2010). Challa & Ravindra (1999) and Venkateshwarlu *et al.,* (2001) reported the allelopathic potential of mango leaves for weed management in rose (Rosa hybrida cv. Happiness) basins. Considering the economic importance of wheat in the economy of Pakistan, the costs of weeds in terms of yield reduction, expenditure on their control and successful utilization of mango allelopathic properties in some crops, it was contemplated in the present study to investigate the feasibility of using mango allelopathy to control or suppress canary grass growth, along with any effect on wheat.

Material and Methods

Extract preparation: Fresh mango (cv. Malda) leaves (old and new) collected from the mango trees (Nuclear Institute for Agriculture and Biology, Faisalabad), were oven dried for 72hrs at 40ºC, and then grinded into powder form. Ten gram of ground plant material was added to 100-ml sterile distilled water in 250-ml flask, wrapped in aluminum foil and stored in low temperature incubator at 25±2 ºC for 72hrs. The extract was then filtered through two layers of cheesecloth, centrifuged (Sigma Centrifuge) at 15000rpm, at 4°C for 20 min and supernatant was collected. This extract concentration (10g/100ml) was considered for future comparisons as 10%. Different dilutions i.e., 7.5, 5, and 2.5% were prepared with sterile distilled water. Sterile distilled water was used as the control. All procedures were carried out in a cross-flow laminar flow cabinet to minimize contamination (Seal *et al*., 2010).

***In vitro* bioassays:** Canary grass and wheat (cv. Sehar) were used as test species in this study. Seeds of canary grass and wheat were surface sterilized with 2% NaClO for 1 min and rinsed three times with sterile distilled water. Then placed in 9-cm Petri plates lined with filter paper (Whatman #1). This had been moistened with 4ml sterile distilled water. The plates were placed in an incubator with a 14 h day/10 h night cycle till germination.

In order to check the allelopathic effect on germinated seeds of test species, 4ml of each extract concentration (both new and old mango leaves) were added to each Petri plate containing germinated seeds of canary grass and wheat, separately. Three replicates were arranged in a completely randomized block design to eliminate the effect of any contamination which can reduce the seed germination under the experimental conditions mentioned above. Shoot and root length was noted 10 days after sowing (DAS). Dry weight of germinated plants was noted after oven drying at 40°C till constant weight. Bioassays were also conducted with seeds that had not been pre-germinated. In this case, seeds were surface sterilized as described above, but were placed immediately into the Petri plates with the 4ml test solutions. Final seeds germinated along with shoot length, root length and dry weight (as described above) were measured 10 days after germination.

***In vivo* bioassays:** Five seeds of wheat and canary grass were sown together in sterilized soil filled plastic pot (30 x 20cm) containing 5 kg soil with three replicates. Pots were irrigated with water and placed in growth room under artificial light at 24 ± 2ºC. Ten percent extract of each new and old mango leaves was used @ 15ml per pot separately. Extract was applied in three ways i.e., one set was treated before germination, second was treated after germination and third was treated before as well as after germination. One spray was applied for pre-emergence and three sprays for post-emergence with the interval of one week. Final germination was recorded till constant germination both in canary grass and wheat. Plant height and shoot dry weight of canary grass and wheat was recorded 120 DAS.

Solvent extraction and fractionation: Using the aforementioned extract preparation method, 100 ml of 10% old and new mango leaves extract was obtained, separately. To precipitate out any proteinaceous material, 300ml of acetone were added to the extracts and then placed for 48 h at 4ºC. The precipitate was filtered through two layers of Whatman #1 filter paper and the acetone evaporated off under reduced pressure, using a rotary evaporator (Buchi, Switzerland). The aqueous extracts were washed with n-hexane to remove green colour. One part of remaining aqueous extracts was used for total phenol determination and second for extraction with ethyl acetate. For this purpose the extracts were partitioned four times with 46 ml of ethyl acetate. Pooled solvent fractions were rotary evaporated under vacuum at a maximum temperature of 40ºC and blown down to dryness with nitrogen. The residues were dissolved in HPLC/LCMS grade methanol. Aqueous layers remained after the removal of ethyl acetate were lyophilized using Freeze Dryer (Alpha I-5, Cherist). The lyophilized material was dissolved in HPLC/LCMS grade methanol. All the methanolic solutions were passed through a C_{18} solid-phase extraction cartridge which had been preconditioned with methanol and 0.1% HCl, respectively, and stored at 4ºC prior to use for Mass Spectrometry and HPLC analysis.

Phenolic contents: Phenolic contents were determined using Folin-Ciocalteu reagent according to Sarwar *et al*., (2001). Chlorogenic acid was used as standard for preparing the calibration curve. Four ml of 4% Na_2CO_3 was added into 200 µl of the samples of mango extracts, Chlorogenic acid and blank, respectively. Thereafter, 200µl Folin-Ciocalteau reagents were added during shaking on vortex mixture. After 30 min incubation at room temperature, the absorbance of the samples and standards was measured at 750 nm against a blank by Double Beam Spectrophotometer (Hitachi U-2800). All determinations were performed in triplicate.

Mass spectrometry: Mass analysis was carried out on LTQ XL Linear Ion Trap quadruple (Thermo Fisher Scientific) instrument. Instrument conditions used were ESI Negative ion mode with eletrospray high voltage of 4.00kv, sheath gas flow 35 units, Auxiliary gas 3.0 units, capillary temperature 275ºC and capillary voltage 30V. Ethyl acetate fraction of new and old mango leaves aqueous extract diluted in methanol was run by direct infusion at a rate of 5ul/min. Spectra were scanned in the range of m/z 50-600 in full scan and the ion isolation width in ion isolation mode was m/z= 5-10.

High performance liquid chromatography (HPLC): On the basis of Mass spectrum and Bioassay study old mango leaves extract was selected for the identification and confirmation of phenolic compounds through HPLC. Identification of phenolic compounds was performed by chromatographic comparison of retention times with standard compounds i.e. 4-hydroxybenzaldehyde, *m*-coumaric, *p*-coumaric, 4-hydroxy benzoic, vanillic, caffeic, gallic and protocatechuic acids etc. (Sigma Chemical Company, USA). Chromatographic separations were made on Varian HPLC, equipped with a Varian pumps and UV Detector at 280 nm. A 20 µl of each methanolic solution of samples and standards was injected onto a reverse phase Thermo Hypersil C-18 column (250×4.6 mm, 5µm particle size). Eluent consisted of an isocratic mixture of water, acetonitrile and acetic acid (88:10:2) over 55 min at flow rate of 1ml/min. The solvents used were of HPLC grade.

Statistical analysis: Data were analyzed by ANOVA and multiple comparison among means was made using least significance difference (LSD) test at $p<0.05$.

Results and Discussion

***In vitro* bioassays:** All the concentrations of aqueous extracts of old as well as new mango leaves showed profound inhibitory effect (15-96%) for the four tested parameters (germination, dry weight, shoot and root growth) on canary grass (Table 1). The highest activity was shown by 10% extract of old mango leaves, with 93-96% inhibition, on all the tested parameters. In case of wheat, the effect of all the concentrations of old mango leaves extract was non significant for all the parameters studied in this experiment. While the new mango leaves extract inhibited the wheat shoot length (4-36%) and root growth (1-21%) and also the dry weight (23-30%) of wheat, under all concentrations. However, it was unable to inhibit or promote the final germination of wheat. It was also noted that the effect of extracts was dependant upon the concentration, greater concentration showed the greater inhibitory effect and vice versa.

Table 1. Phytotoxic effect of different concentrations of aqueous extract of new and old mango leaves on germination and early seedling growth parameters of canary grass and wheat.

Extracts	Final germination (%)		Shoot length/plant (cm)		Root length/plant (cm)		Dry weight (mg)	
	NL	OL	NL	OL	NL	OL	NL	OL
	Canary grass							
Control	73 ± 3.33a	76.66 ± 3.33a	3.11 ± 0.11a	4.13 ± 0.005a	4.13 ± 0.08a	4.52 ± 0.12a	7.00 ± 0.51a	7.33 ± 0.33a
2.5%	27 ± 3.33b (63)	13.33 ± 5.78b (82)	2.63 ± 0.36ab (15)	2.3 ± 0.76ab (43)	1.77 ± 0.29b (57)	0.86 ± 0.17b (80)	3.66 ± 0.33b (47)	1.33 ± 0.56b (81)
5.0%	13 ± 3.33b (82)	13.33 ± 5.78b (82)	1.58 ± 0.16bc (49)	0.83 ± 0.14b (79)	1.33 ± 0.16bc (67)	0.66 ± 0.00b (85)	1.33 ± 0.33c (81)	1.66 ± 0.76b (77)
7.5%	13 ± 3.33b (82)	13.33 ± 0.00b(82)	0.79 ± 0.04cd (74)	0.66 ± 0.00b (84)	0.83 ± 0.14bc (79)	0.50 ± 0.14b (89)	1.00 ± 0.33c (85)	1.00 ± 0.33b (86)
10%	10 ± 3.33b (86)	3.33 ± 3.33b (95)	0.38 ± 0.04d (87)	0.16 ± 0.16b (96)	0.387 ± 0.06c (90)	0.33 ± 0.33b (93)	1.00 ± 0.33c (85)	0.33 ± 0.33b(95)
	Wheat							
Control	100 ± 0.00^{NS}	100 ±0.00 ^{NS}	9.06 ± 0.36 a	6.90 ± 0.05 ^{NS}	9.23 ± 0.23 a	7.93 ± 0.03 ^{NS}	173 ± 2.52 a	173 ± 2.52^{NS}
2.5%	100 ± 0.00 (0)	100 ± 0.00 (0)	8.66 ± 0.06a (4)	6.80 ± 0.11(1)	9.20 ± 0.30 a (0)	7.83 ± 0.12 (1)	133 ± 15.6 bc (23)	170 ± 0.66 (1)
5.0%	100 ± 0.00(0)	100 ± 0.00 (0)	7.93 ± 0.35 a (12)	6.80 ± 0.11(1)	9.06 ± 0.33 a (1)	7.80 ± 0.05 (1)	163 ± 6.81ab (5)	175 ± 1.15 (-1)
7.5%	97 ± 3.33 (3)	100 ± 0.00(0)	6.26 ± 0.06 b (30)	6.80 ± 0.09(1)	7.86 ± 0.40 b (14)	7.80 ± 0.11 (1)	129 ± 12.1bc (25)	163 ± 6.67 (5)
10%	97 ± 3.33(3)	96 ± 3.33(4)	5.73 ± 0.67 b (36)	6.80 ± 0.11(1)	7.2 ± 0.20 b (21)	7.90 ± 0.05 (0)	120 ± 2.52c (30)	173 ± 1.33 (0)

NL= New leaves; OL= Old leaves; values are mean ± standard error followed by letters imply the significant differences ($p<0.05$) between the values in the same column; values + and - in the parentheses shows the percent inhibition and stimulation over control, respectively; NS= Non-significant

Similar effects as described above were observed when the extracts were used against the pre-germinated seeds of canary grass (Table 2). New mango leaves extract inhibited the shoot length, root length and dry weight of canary grass by 27-50%, 32-43% and 0-44% respectively. Old mango leaves extract inhibited the shoot length, root length and dry weight upto 58, 56 and 51%, respectively. While none of the extracts showed inhibition to either dry weight, root and shoot growth of pre-germinated seeds of wheat except that old mango leaves extract which significantly increased the dry weight of wheat up to 17%.

***In vivo* bioassays:** When the phytotoxic potential of aqueous extract of new and old mango leaves was checked against the germination and growth parameters of test species in pot experiment, it was noted that new leaves had non-significant effect on the germination of canary grass either applied before or in combination of both stages, while old mango leaves inhibited the germination upto 50%. Inhibition to shoot growth (21.5%) and dry weight (66%) was noted when the old mango leaves extract was applied before germination. Germination parameter in wheat was found to be non significant. However, shoot length and dry weight were enhanced by old mango leaves extract while new mango leaves inhibited these parameters, generally dependent upon the stage of application. One exception was found in case of old leaf extract when applied after emergence; it reduced 13% dry biomass of wheat (Table 3).

Phenolic contents: New leaves showed higher phenolic content (172.3 mg g^{-1} dry weight) than the old ones (134.2 mg g^{-1} dry weight).

Mass spectrometry: ESI-Negative mode mass spectrum, showed a number of molecular ion peaks at different m/z values, some of them are Ellagic acid/Quercetin $[M-H]^-$ =301, Caffeic acid $[M-H]^-$=179, Coumaric acid $[M-H]^-$ =163, Ferulic acid $[M-H]^-$=193, Sinapic acid $[M-H]^-$=223, Vanillic acid $[M-H]^-$=167, Protocatechuic acid $[M-H]^-$ =153, Chlorogenic acid $[M-H]^-$=353, Syringic acid $[M-H]^-$=197, Catechol $[M-H]^-$=109, Pyrogallol $[M-H]^-$=125, Hydroxybenzaldehyde $[M-H]^-$= 121, Gallic acid $[M-H]^-$ =169, Hydroxy benzoic acid $[M-H]^-$=137, Vanillin $[M-H]^-$ =151 and Catechin $[M-H]^-$=289. These peaks were identical in both the methanolic solutions of ethyl acetate fraction of new and old mango leaves (Figs. 1 & 2).

High performance liquid chromatography: HPLC indicated the presence of 4-hydroxybenzaldehyde, *m*-coumaric, *p*-coumaric, 4-hydroxy benzoic, vanillic, caffeic, gallic and protocatechuic acids with retention times 13.7, 35.7, 20.46, 9.47, 11.1, 11.2, 4.3 and 6.1, respectively in the methanolic solutions of ethyl acetate layer and lyophilized material of old mango leaves (Figs. 3 & 4). Due to lack of standards, many different peaks remained unidentified.

Discussion

According to scientific research, the use of large amount of chemical pesticides poses health and safety risks, and is environment unfriendly (Ronald, 2000). Meanwhile, various parts of plants in powder or in aqueous extract have been reported as a source of naturally occurring and selective herbicides (Jamil *et al*., 2009; El-Rokiek *et al*., 2010). Hence the use of synthetic pesticides is tending to decrease and needs replacement with other safer compounds. Therefore, the search for non-toxic natural herbicides is of increasing interest.

Mango is one of the abundant trees in Pakistan, the leaf material of which can be another source of natural herbicide, as it has capacity of killing or suppressing weed growth (Rudramuni *et al*., 2006; El-Rokiek *et al*., 2010). The results illustrated in present investigation revealed mango leaves extract not only induced significant reduction in germination, early seedling growth and yield of canary grass *In vitro* or *In vivo*, but also showed moderate stimulatory effect on the germination and growth of wheat. The effect of old mango leaves was better as compared to new ones, because new mango leaves also induced reduction in dry weight and grain weight of wheat. It was also noted that the effect of extracts was dependant upon the concentration. Greater concentration showed the greater inhibitory effect and vice versa.

Mango leaves have been reported to contain many different kinds of phenolic compounds (El-Rokiek *et al*., 2010). The determination of individual phenolic compounds by HPLC is of complicated performance and presented only 50-60% of total phenolic content (Scalbert & Williamson, 2000). In our study, Folin-Ciocalteu method used to compare the total phenolic content showed high phenolic content in new leaves than the old ones. These results are in accordance to the findings of Nantitanon *et al*., (2010) they reported that young guava leaves possessed the highest phenolic contents followed by that of old leaves and middle age leaves, respectively. As described above, new mango leaves also showed phytotoxic effect against wheat, this might be due to the greater concentration of those phenolic compounds which are selectively more phytotoxic to wheat.

Polyphenolics is a highly inclusive term that covers many different subgroups of simple phenols, phenolic acids and flavonoids; and is ubiquitous in land plants (Tsao & Deng, 2004). Phenolic compounds are important intermediates in the formation of humus (Haider *et al*., 1975) and are, in part, responsible for stabilizing nitrogen in organic forms in soils (Parsons & Tinsley, 1975) and may responsible for increase in production of crops. In our study the mango leaves extract moderately enhanced the growth of wheat. Phenolic compounds are also selectively toxic to plants (Macias *et al*., 1999). The identification of phenolic acids, including p-hydroxybenzoic, vanillic, p-coumaric and ferulic acids, in plant extracts (Chon & Kim, 2004) and decomposing plant residues (An *et al*., 2001) and soils (Whitehead *et al*., 1983) has led to the suggestion that they might be involved in allelopathic effects between competing plant species. So, there is a great need to identify these compounds. In this concern we have identified phenolic compounds including 4-hydroxybenzaldehyde, *m*-coumaric, *p*-coumaric, 4-hydroxy benzoic, vanillic, caffeic, gallic and protocatechuic acids in mango leaves.

Table 2. Phytotoxic effect of different concentrations of aqueous extract of new and old mango leaves on early growth parameters of pre-germinated seeds of canary grass and wheat.

Extracts	Shoot length/plant (cm)		Root length/plant (cm)		Dry weight/treatment (mg)	
	NL	OL	NL	OL	NL	OL
	Canary grass					
Control	3.10 ± 0.09 a	3.10 ± 0.09 a	3.26 ± 0.17 a	3.26 ± 0.17 a	8.33 ± 0.33 a	8.33 ± 0.33 a
2.5%	2.27 ± 0.11 b (27)	1.80 ± 0.14 b (42)	2.18 ± 0.10 b (33)	2.00 ± 0.13 b (38)	6.58 ± 0.31 a (21)	5.41 ± 0.50 b (35)
5.0%	2.26 ± 0.13 b (27)	1.86 ± 0.06 b (55)	2.20 ± 0.11 b (32)	2.10 ± 0.09 b (35)	6.66 ± 0.33 a (20)	5.66 ± 0.66 b (32)
7.5%	1.73 ± 0.16 c (44)	1.36 ± 0.20 c (56)	1.96 ± 0.03 b (39)	1.73 ± 0.08 bc (46)	4.66 ± 0.33 b (44)	4.66 ± 0.33 b (44)
10%	1.53 ± 0.16 c (50)	1.30 ± 0.14 c (58)	1.83 ± 0.03 b (43)	1.43 ± 0.08 c (56)	8.33 ± 0.91 a (0)	4.00 ± 0.57 b (51)
	Wheat					
Control	4.25 ± 2.45 ^{NS}	4.25 ± 0.52 ^{NS}	4.33 ± 2.50 ^{NS}	4.33 ± 0.30 ^{NS}	58.00 ± 6.93a	58.00 ± 6.93 ^{NS}
2.5%	3.53 ± 2.04(17)	4.80 ± 0.11(-12)	3.93 ± 2.27(9)	4.53 ± 0.13 (-4)	44.33 ± 2.96b (23)	68.00 ± 3.5 (-17)
5.0%	4.06 ± 2.34(4)	4.73 ± 0.17(-11)	4.33 ± 2.50 (0)	4.33 ± 0.24 (0)	58.66 ± 1.76a (-1)	57.00 ± 7.52 (1)
7.5%	3.53 ± 2.04(17)	4.40 ± 0.23(-3)	3.66 ± 2.11(15)	4.20 ± 0.30 (3)	44.00 ± 2.31b (24)	64.66 ± 3.84 (-10)
10%	4.33 ± 2.50(-1)	4.33 ± 0.17(-1)	3.53 ± 2.04 (18)	4.33 ± 0.17 (0)	55.00 ± 2.52ab (5)	58.33 ± 6.77 (0)

NL= New leaves; OL= Old leaves; values are mean ± standard error followed by letters imply the Significant differences ($p<0.05$) between the values in the same column; values + and - in the parentheses shows the percent inhibition and stimulation over control, respectively; NS= Non-significant.

Table 3. Phytotoxic effect of different concentrations of aqueous extract of new and old mango leaves on germination and growth parameters of canary grass and wheat in pots.

Extracts	Germination percentage		Shoot length/plant (cm)		Shoot dry weight/treatment (g)	
	NL	OL	NL	OL	NL	OL
	Canary grass					
Control	86.66 ± 6.67 ^{NS}	93.33 ± 6.67 a	26.66 ± 1.45 b	27.19 ± 1.24 a	1.98 ± 0.01 a	2.10 ± 0.02 a
BE	73.33 ± 6.67 (15)	46.66 ± 6.67 b (50)	21.33 ± 0.66 c (20)	21.33 ± 0.66 b (21.5)	1.75 ± 0.05 b (11)	0.72 ± 0.005 c (66)
AE	–	–	21.00 ± 0.57 c (21)	26.00 ± 0.57 a (4)	1.69 ± 0.05 b (15)	1.52 ± 0.04 b(28)
Both	86.66 ± 6.67 (0)	53.33 ± 6.67 b (43)	32.33 ± 1.45 a (-21)	21.33 ± 0.88 b (21.5)	2.11 ± 0.07 a (-6)	0.79 ± 0.04 c (63)
	Wheat					
Control	86.66 ± 6.67 ^{NS}	73.33 ± 6.67 ^{NS}	36.00 ± 1.00 b	34.66 ± 0.88 b	2.80 ± 0.06 a	2.56 ± 0.05 c
BE	80.00 ± 0.00 (7)	93.33 ± 6.67 (-27)	40.66 ± 0.33 a (-13)	41.66 ± 0.33 a (-20)	2.77 ± 0.08 a (1)	2.89 ± 0.01 a (-13)
AE	–	–	41.33 ± 0.33 a (-14)	41.00 ± 0.57 a (-18)	2.36 ± 0.13 b (16)	2.24 ± 0.05 d (13)
Both	73.33 ± 6.67 (15)	93.33 ± 6.67 (-27)	39.66 ± 0.33 a (-6.6)	42.66 ± 0.66 a (-23)	2.24 ± 0.04 b (20)	2.73 ± 0.04 b (-6)

NL= New leaves; OL= Old leaves; BE= treatment applied before emergence; AE= treatment applied after emergence; Both= treatment applied before as well as after emergence; values are mean ± standard error followed by letters imply the significant differences ($p<0.05$) between the values in the same column; values + and - in the parentheses shows the percent inhibition and stimulation over control, respectively; NS= Non-significant, - = Not-observed

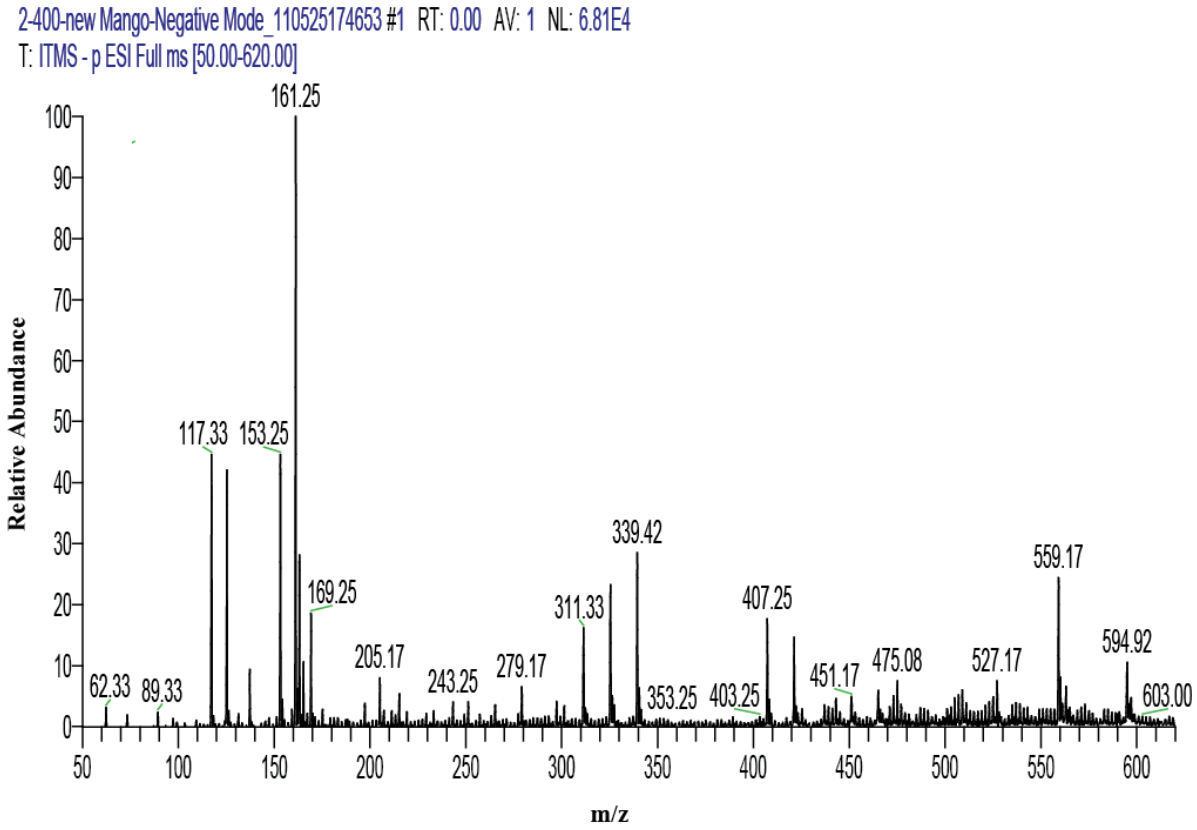

Fig. 1. Mass spectrum of methanolic solution of ethyl acetate layer of new mango leaves aqueous extarct.

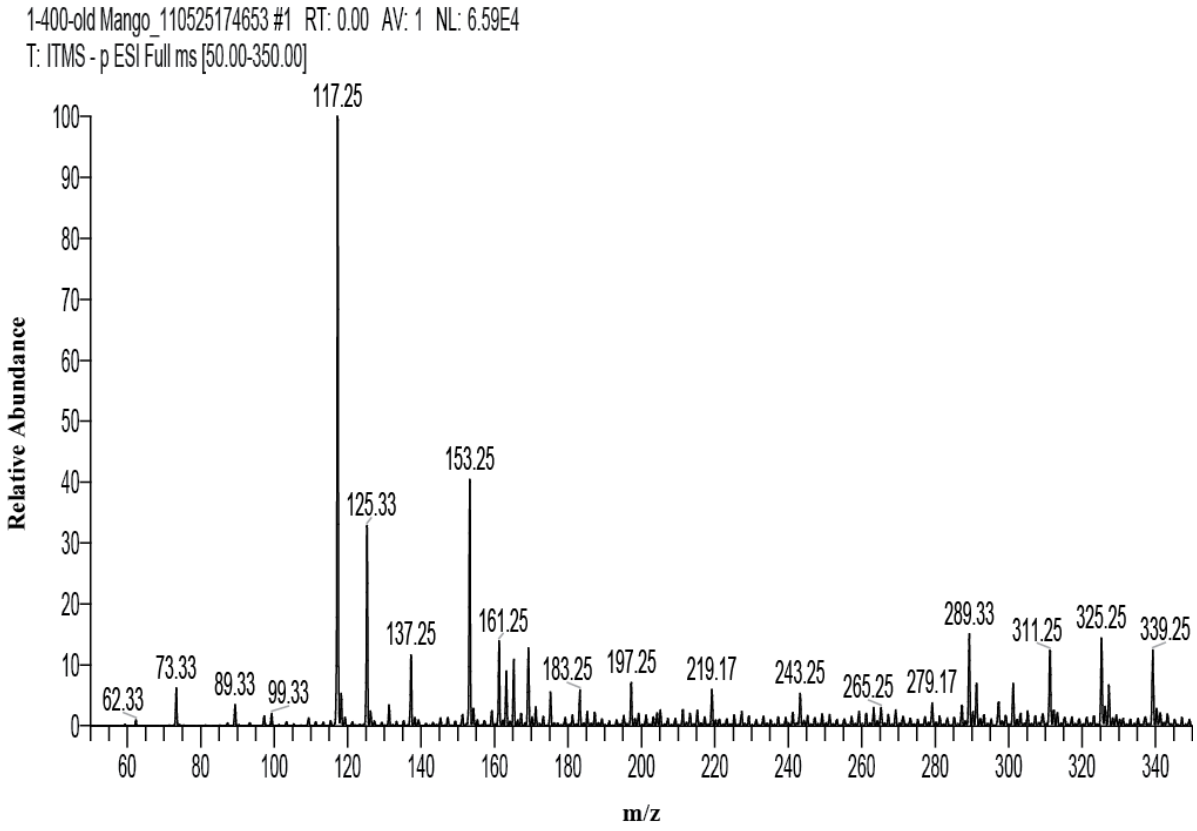

Fig. 2. Mass spectrum of methanolic solution of ethyl acetate layer of old mango leaves aqueous extarct.

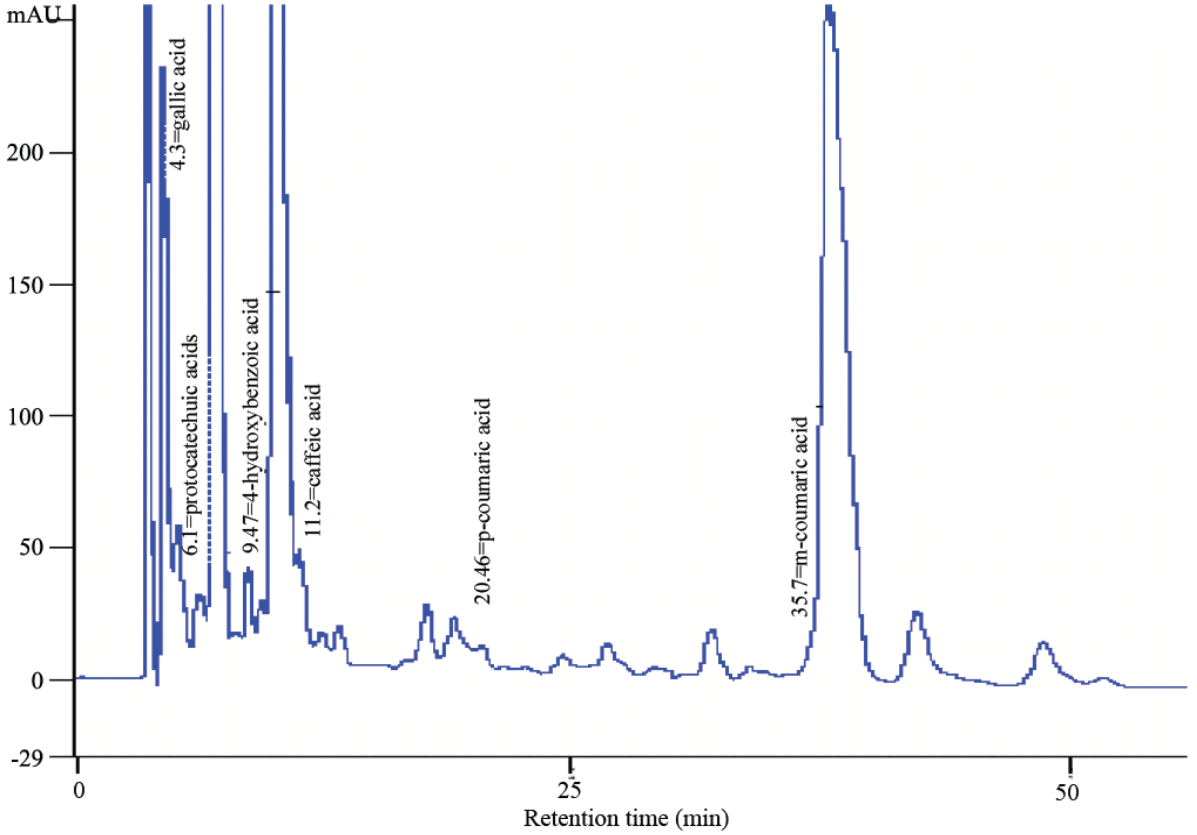

Fig. 3. HPLC chromatogram of methanolic solution of ethyl acetate layer of old mango leaves aqueous extract.

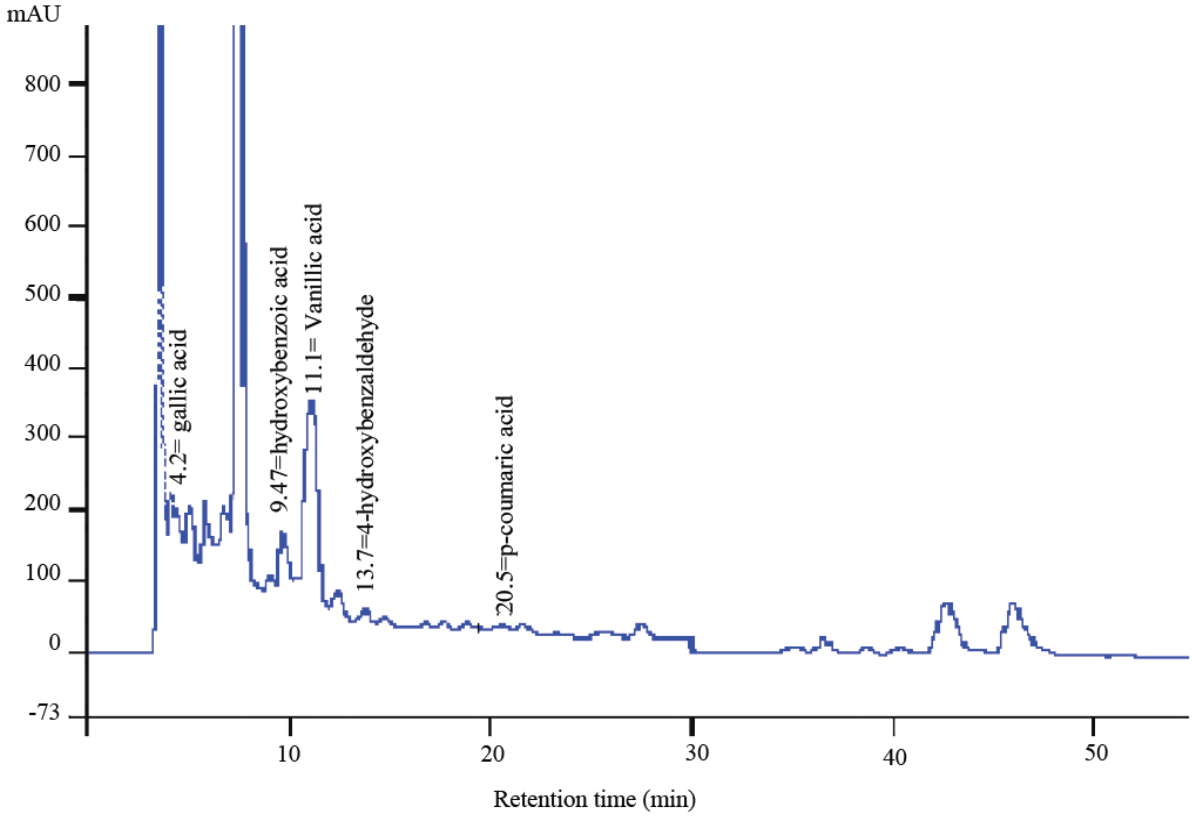

Fig. 4. HPLC chromatogram of methanolic solution of lyophilized layer of old mango leaves aqueous extract.

Conclusion

It has been concluded from present study that mango leaves could be used as a parallel safety tools to suppress canary grass and to enhance wheat growth. However, further research is needed to optimize the concentration of mango leaves extract to achieve the maximum inhibition of canary grass and to compare with existing synthetic herbicides. In addition to this, identification of the unidentified peaks present in HPLC-chromatogram and MS-spectrum, in mango leaves extract may also be brought under consideration to determine more inhibitory compounds.

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