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# Allelopathic potential of 73 weed species in Pakistan

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**ABSTRACT. Introduction:** Use of allelochemicals as inexpensive, biodegradable and eco-friendly substitutes for the management of weeds in crops is a central topic nowadays. **Objective:** The current study represented the comprehensive screening of allelopathic activity of 73 weeds in Pakistan by the Sandwich method and dish pack method. To investigate the allelochemical compound in top most allelopathic weed. **Methods:** Allelopathic effects of selected weeds leaves leachate were determined through sandwich technique, while volatile allelopathic effects through dish pack techniques. Qualitative and quantitative phytochemical techniques were applied to investigate allelochemical potential of *Melilotus indicus*. The statistical analysis of the data described the allelopathic effect of 73 weed plants on lettuce seedling growth in terms of radicle and hypocotyl elongation. **Results:** Elongation percentage of radicle and hypocotyl ranged 0-74 % to 0-148 % and 0-75 % to 0-84 % respectively at 10 mg concentration of dry plant powder in sandwich method while it was 2-234 % and 7-150 % at in Dish Pack method. Among the plants screened for phytotoxic activity, *M. indicus* contained the strongest allelochemicals. Qualitative and quantitative analysis of *M. indicus* showed the presence of flavonoids and phenolic compound along with other allelochemical. **Conclusions:** Selected weeds may have strong allelochemical potential that can help in the development of bioactive compounds from plant species to be used as natural herbicides and pesticides for sustainable management of weeds and pest.

**Key words:** bioassay, phytochemicals, allelopathy, sandwich method, dish pack method.

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In Pakistan, the wheat crop is facing competition for nutrition and space with some major weeds like *Chenopodium album*, *Avena fatua, Euphorbia helioscopia, Rumex dentatus* and *Phalaris minor* (Ali, Tahir, Shah, Khan, & Mehmood, 2017). An annual loss of ~28 billion Pakistani Rupees is estimated due to weed infestation in wheat crop in Pakistan (Khan et al., 2018). To supplement the nutrient requirements and weed control, heavy doses of synthetic chemicals (herbicides) are used. However, resistance is developing in different weeds by continuous application of huge quantities of synthetic chemicals that are also a serious threat to the environment. To avoid crop loss by weeds and assure a healthy

environment, organic weed control measures must be adopted (Fisher et al., 2012; Khan et al., 2018). The demand for organic products has been increased during the last decades (Anwar et al., 2019).

The growth and development of plants are affected by allelochemicals. Allelochemicals isolated from plants could substitute synthetic agrochemicals (Bhadoria, 2011; Appiah et al., 2015). In crop production, weed management through allelopathy is beneficial and environmentally friendly. Allelochemicals have shorter half-lives with different chemical structures and diverse modes of action (Appiah et al., 2015; Abbas et al., 2018). A diverse flora of about 6 000 vascular plants including weeds of agro-ecosystem are reported from Pakistan (Shinwari, Shinwari, & Fujii, 2013). Various allelochemicals are being released to the environment by these plants. The investigation and exploration of these bio-compounds can be carried out for natural herbicides to increase crop yield as well as avoid negative impacts of synthetic chemicals on the environment. The current research focused on screening of allelopathic/herbicidal potential in 73 selected plants for natural weed control strategy through allelopathy.

## MATERIALS AND METHODS

**Collection of plant materials:** The fresh leaves of 73 weeds were collected from fields, roadsides, wastelands and meadows of Pakistan which were later dried in oven (Biobase) at 45 °C for approximately 24 h. A milling machine was used for grinding dried leaves in powder form.

**Allelopathic screening of selected medicinal weeds:** Sandwich and Dish pack screening methods were applied to determine the allelopathic potential of selected medicinal weeds

**Sandwich method:** For screening the allelopathic potential of selected plant's leaves leachates, the sandwich method was adopted (Fujii et al., 2004) with lettuce (*Lactuca sativa*) seeds because it is reported earlier that lettuce seeds are reliable for germination and susceptible to chemicals. Agar growth medium is best for seedling germination of lettuce seeds (Shinwari et al., 2013). Growth medium of 7.5 g agar per 1 000 mL of distilled water was prepared. Selected weed species were screened out in three replicates and mean of the replications (± SE) was presented in data. The untreated agar in multi-well without plant materials was set as control. The multi-wells were sealed airtight with plastic tap and wrapped in aluminum foil. Then multi-well plates were placed in an incubator (Biobase Model BJPX-HI10) for 72 h at 25 °C. The measurement of radicle and hypocotyl were taken with the help of tweezers and graph papers. Percentage inhibition of radicle and hypocotyl were calculated as:

$$\% \text{ Inhibition} = \frac{(\text{Average length of treatment radicle/hypocotyl})}{(\text{Average length of control radicle/hypocotyl})}$$

**Dish pack method:** For screening the allelopathic potential of selected plants, dish pack method was adopted on lettuce seeds (Fujii, 2005). Multi-dish plastic plates (6 well; 36 × 18 mm each) were used for analysis. The distances from the center of the source well (where plant sample was placed) to the center of other wells were 41, 58, 82, and 92 mm respectively. Oven-dried leaf litter (100 mg) was placed in the source well while in other wells of multi-dish, filter papers were laid and 7 mL distilled water was added to each well. A respective control was maintained without plant material in the source well. Seven seeds of lettuce were placed on the filter paper of each well. The multi-wells placed were sealed airtight with cellophane tape and wrapped in aluminum foil. The dishes were then placed in an incubator at 25 °C (Biobase Model BJPX-HI10) for three days. The length of radicle and

hypocotyl were measured and compared to control to show the degree of inhibition.

The experiment was designed using three randomized replicates and the mean of them was reported. In both Dish pack and Sandwich methods for each plant species; the mean and standard deviation (SD) and the standard deviation variance (SDV) was calculated to determine the inhibition pattern of radicles and hypocotyl of the lettuce seedlings (Shinwari & Fujii, 2013).

**Qualitative analysis:** Qualitative compound analysis of top allelopathic weed *Melilotus indicus* crude methanolic extract was carried out for the identification of different allelochemicals classes.

**Phenols assessment:** The formerly described technique (Richardson, 1990; Majid et al., 2015) was followed for the identification of phenols in *M. indicus*. Each sample (1 mg) was suspended in 2 ml of distilled water containing 10 % ferric chloride. Change of suspension to blue or green color showed the presence of phenol.

**Flavonoids assessment:** For identification of flavonoids in the selected plants samples, the Trease and Evans (1989) protocols were adopted. Briefly, 1 ml of 2 N sodium hydroxide reacted with 1 mg of sample. A color change to yellow confirmed the presence of flavonoid in *M. indicus*.

**Coumarins assessment:** An aliquot sample 1 mg/ml and 1 ml of 10 % NaOH was mixed. The appearance of yellow color as a result of a reaction in the test tube indicated coumarins in the test sample (Richardson, 1990).

**Saponins assessment:** The suspension was formed by mixing of 2 mg of sample in 2 ml distilled water upon vigorous shaking. Formation of a soapy layer of almost 1-2 cm indicated the presence of saponins (Richardson, 1990).

**Tannins assessment:** Mixing of 1 mg sample with 2 ml of 5 % ferric chloride gave dark blue or greenish black color which is the confirmative sign of tannins (Trease & Evans, 1989).

**Terpenoids assessment:** 0.5 mg of sample was added in 2 ml of each chloroform and concentrated sulphuric acid that formed the red-brown layer and confirmed the presence of terpenoids (Trease & Evans, 1989).

**Anthraquinone assessment:** 1 mg sample was mixed with 2 ml of diluted 2 % hydrochloric acid, red color was developed that confirmed anthraquinones (Richardson, 1990).

**Anthocyanin and betacyanin assessment:** 1 mg plant sample was dissolved in 2 ml of 01 N NaOH and boiled for 10 min. The formation of bluish-green color indicated the anthocyanin and yellow color showed betacyanin presence (Trease & Evans, 1989).

**Alkaloids assessment:** The reaction of concentrated Sulphuric acid and 2 mg of the selected sample with Mayer's reagent showed green color or white precipitates that confirmed the presence of alkaloid (Trease & Evans, 1989).

**Total phenolic contents (TPC):** Total phenolic contents were determined by spectrophotometer (Kim, Jeong, & Lee, 2003). The sample was prepared in a volumetric bottle (25 ml) by adding 1 ml plant content in 9 ml of deionized water followed by the addition of 1 ml phenol, and 10 ml of 7 % sodium carbonate and pure deionized water until the final volume of 25 ml. The optical density was measured at a wavelength of 750 nm using Gallic acid as a standard.

**Total flavonoid contents (TFC):** The spectrophotometric technique was applied to quantify total flavonoid content (Park et al., 2008). The reaction mixture was prepared

in a test tube containing 0.3 mL plant content, 0.15 mL of 0.5 mol/L $NaNO_2$, 0.3 M $AlCl_3$. $6H_2O$ and 3.4 ml of 30 % methanol. After 5 min, 1 ml of NaOH was added and optical density was measured at 506 nm using rutin standard.

## RESULTS

A total of 73 plant species were studied for their allelopathic potential. Using the sandwich method, *Melilotus indicus* was identified as the strongest allelopathic plant among the 73 weed species, followed by *Medicago parviflora*. *M. indicus* showed strong inhibition on hypocotyl and radicle elongation of lettuce seeds (Fig. 1). The statistical analysis of the Sandwich data is represented in table 1, which described the allelopathic effect of leachates of 73 weeds plants on lettuce seedling elongation (radicle and hypocotyl percentage elongation). It is evident that elongation percentage of radicle and hypocotyl ranged 0-74 % and 0-148 % (10 mg) respectively in Sandwich

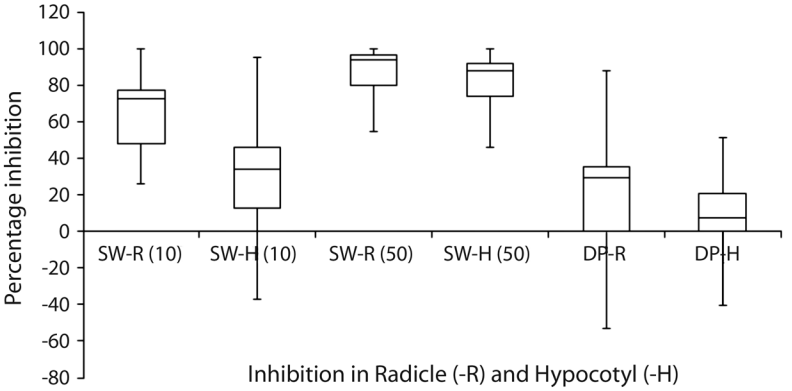


**Fig. 1.** Range of Percentage Inhibition in Radicle (-R) and Hypocotyl (-H) of 73 Weed Species by Sandwich (SW).

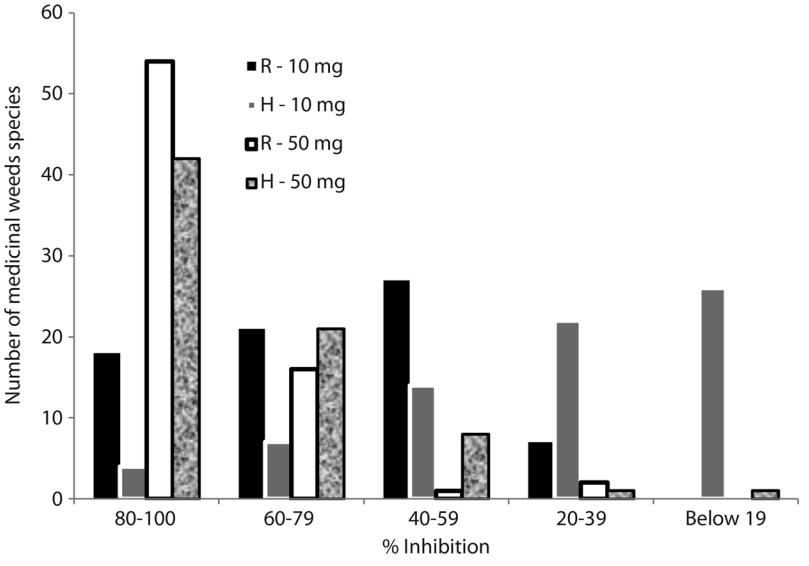


**Fig. 2.** Frequency Distribution of Percentage Inhibition among medicinal weeds through sandwich method.

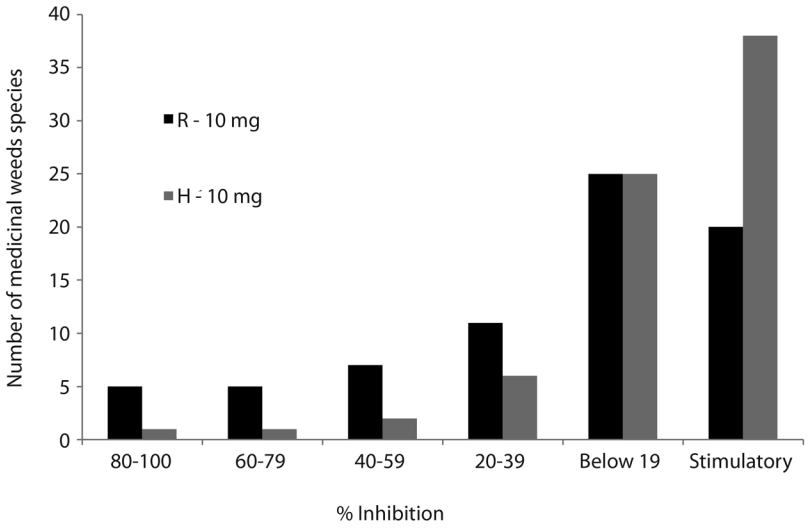


**Fig. 3.** Frequency distribution of percentage inhibition among medicinal weeds through dish pack method.

method (Table 1, Fig. 2) while 2-234 % and 7-150 % in Dish Pack method (Table 2, Fig. 3) compared to control. Sandwich method data of inhibition of radicle at 10 mg concentration is categorized into 5 classes (Table 1). Complete inhibition in the first group (80-100 %) was shown by *Melilotus indicus* and *Medicago parviflora*. Strongest inhibitory activity i.e., 90-99 % was observed in 4 species (*Melilotus alba, Peganum harmala, Coronopus didymus* and *Nasturtium officinale*). 80-86 % inhibition was exhibited by *Taverniera cuneifolia, Crotalaria medicaginea, Solanum nigrum, Urtica dioica, Achyranthes aspera, Coriandrum sativum, Otostegia limbata, Avena fatua, Parthenium hysterophorus, Cirsium arvense, Xanthium strumarium,* and *Anagallis arvensis*. The lowest inhibitory activity in this study was observed sizes in the range of 20-39 % by *Helianthus annuus, Lythrum salicaria, Dichanthium annulatum, Salvia moocroftiana, Phalaris minor, Cyperus* sp., and *Saxifraga rotundifolia*. Members of Papilionaceae were ranked the strongest inhibitory plant species among the evaluated samples using the sandwich method. Overall there were 8 species from papilionaceae out of which 5 exhibited 80-100 % inhibition while 3 others showed 60-79 % inhibition of radicle growth (Table 1).

In dish pack method (Table 2), *Coronopus didymus* ranked at the top with 98 % inhibition rate of radical. Next 4 plants with strong inhibition are in the range of 86-93 % i.e., *Melilotus alba, Melilotus indicus*, *Malva parviflora* and *Solanum nigrum*, *Nasturtium officinale*, *Urtica dioica*, *Cyperus* sp., *Vicia sativa*, *Medicago parviflora*, comes next in the ranking showing 60-79 % inhibition in radical growth rate. *Achyranthes aspera, Crotalaria medicaginea, Amaranthus viridis, Xanthium strumarium, Phalaris minor, Avena fatua, Oxalis corymbos* showed 40-59 %. A total of 39 plant species showed relatively weak inhibitory potential against the control plant species. 20 plant species exhibited stimulatory potential i.e., *Verbena tenuisecta, Saxifraga rotundifolia, Salvia aegyptiaca, Otostegia limbata, Sida alba, Peganum harmala, Oxalis corniculata, Euphorbia hirta, Rumex nepalensis, Adiantum caperis-veneris, Typha minima, Oenothera rosea, Solanum erianthum, Potamogeton lucens, Lythrum salicaria, Cissampelos pareira, Aloe vera, Anisomeles indica, Euphorbia helioscopia* and *Vernonia anthelmintica*. *Euphorbia helioscopia* and *Vernonia anthelmintica* remain conspicuous by having more than 200 % elongation rate.

Total phenolic and flavonoid content were identified from the methanolic extract

TABLE 1
Evaluation of allelopathic activity by leaf litter of 73 medicinal weeds species through Sandwich method at the litter concentration of 10 mg

| Botanical Name | Family Name | Growth | |
|---|---|---|---|
| | | Radicle | Hypocotyl |
| Control | | 100.00 | 100.00 |
| *Melilotus indicus* L. | Papilionaceae | 0.00 | 0.00** |
| *Medicago parviflora* E.H.L. | Papilionaceae | 0.00 | 0.00** |
| *Melilotus alba* Desr. | Papilionaceae | 0.82 | 3.41 |
| *Peganum harmala* L. | Nitrariaceae | 5.05 | 30.61 |
| *Coronopus didymus* (L.) | Brassicaceae | 6.57 | 19.39 |
| *Nasturtium officinale* W.T. | Brassicaceae | 7.58 | 24.49 |
| *Anagallis arvensis* L. | Primulaceae | 9.09 | 33.67 |
| *Taverniera cuneifolia* Roth. | Papilionaceae | 13.70 | 33.77 |
| *Crotalaria medicaginea* Lamk. | Papilionacaea | 13.73 | 35.92 |
| *Solanum nigrum* L. | Solanaceae | 14.14 | 45.92 |
| *Urtica dioica* L. | Urticaceae | 15.15 | 48.98 |
| *Achyranthes aspera* L. | Amaranthaceae | 15.66 | 41.84 |
| *Coriandrum sativum* L. | Apiaceae | 16.33 | 51.13 |
| Otostegia limbata Benth. | Lamiaceae | 17.16 | 55.34 |
| *Avena fatua* L. | *Poaceae* | 18.03 | 80.67 |
| Parthenium hysterophorus L. | Asteraceae | 18.11 | 29.55 |
| *Cirsium arvense* (L.) | *Asteraceae* | 18.58 | 82.35 |
| Xanthium strumarium L. | Asteraceae | 19.70 | 50.00 |
| Centaurea iberica T. | Asteraceae | 22.73 | 45.92 |
| *Trichodesma indicum* (L.) | *Boraginaceae* | 24.04 | 67.23 |
| *Digera muricata* (L.) | Amaranthaceae | 24.51 | 70.87 |
| *Solanum erianthum* D Don. | Solanaceae | 24.81 | 54.30 |
| *Helianthus annuus* L. (petals) | Asteraceae | 25.98 | 66.02 |
| *Typha minima* Funck. | Typhaceae | 27.27 | 42.86 |
| *Potamogeton lucens* L. | Potamogetonaceae | 27.40 | 28.57 |
| *Malva parviflora* L. | Malvaceae | 27.76 | 57.14 |
| *Verbena tenuisecta* Briq. | Verbenaceae | 27.78 | 55.10 |
| *Cyperus* sp. | Cyperaceae | 28.79 | 69.39 |
| *Argyrolobium roseum* Camb. | Papilionaceae | 29.09 | 63.10 |
| *Euphorbia hirta* L. | Euphorbiaceae | 30.13 | 71.92 |
| *Oxalis corniculata* L. | Oxalidaceae | 30.81 | 66.33 |
| *Sonchus asper* (L.) | Asteraceae | 33.74 | 67.05 |
| *Pteris cretica* L. | Pteridaceae | 34.31 | 60.19 |
| *Artemisia scoparia* W & K. | Asteraceae | 34.80 | 57.28 |
| *Oxalis corymbosa* DC. | Oxalidaceae | 35.35 | 77.55 |
| *Cannabis sativa* L. | Cannabaceae | 36.07 | 90.76 |
| *Vicia sativa* L. | Papilionacaea | 36.21 | 56.82 |
| *Saussurea heteromalla* D.Don. | Asteraceae | 38.37 | 78.20 |
| *Rhynchosia minima* (L.) | Papilionaceae | 39.71 | 85.44 |
| *Oenothera rosea* L' Her. | Onagraceae | 41.56 | 77.27 |
| *Sida cordata* (Burm.f.) | Malvaceae | 42.16 | 87.38 |
| *Barleria cristata* L. | Acanthaceae | 42.82 | 81.05 |
| *Convolvulus arvensis* L. | Convolvulaceae | 44.36 | 82.12 |

TABLE 1 (Continued)

| Botanical Name | Family Name | Growth | |
|---|---|---|---|
| | | Radicle | Hypocotyl |
| *Taraxacum officinale* L. | Asteraceae | 44.44 | 67.05 |
| *Ipomoea cornea* Mart. | Convolvulaceae | 45.71 | 102.26 |
| *Phalaris aquatica* L. | Poaceae | 45.86 | 75.50 |
| *Commelina benghalensis* L. | Commelinaceae | 46.58 | 76.62 |
| *Adiantum caperis -veneris* L. | Pteridaceae | 47.88 | 79.76 |
| *Vernonia anthelmintica* L. | Asteraceae | 50.21 | 76.14 |
| *Plantago lanceolata* L. | Plantaginaceae | 50.23 | 79.22 |
| *Chenopodium ambrosioides* Briq. | Chenopodiaceae | 50.27 | 87.39 |
| *Carthamus oxyacantha* M. | Asteraceae | 50.61 | 76.69 |
| *Amaranthus viridis* L. | Amaranthaceae | 50.75 | 90.07 |
| *Salvia aegyptiaca* L. | Lamiaceae | 50.91 | 94.05 |
| *Lantana camara* L. | Verbenaceae | 51.85 | 84.09 |
| *Sida alba* L. | Malvaceae | 51.88 | 94.04 |
| *Conyza bonariensis* (L.) | Asteraceae | 52.63 | 78.15 |
| *Rumex nepalensis* Spreng. | Polygonaceae | 54.32 | 88.64 |
| *Nerium oleander* L. | Apocynaceae | 54.55 | 92.86 |
| *Scrophularia altaica* Murray. | Scrophulariaceae | 54.79 | 57.14 |
| *Euphorbia helioscopia* L. | Euphorbiaceae | 56.33 | 93.23 |
| *Cissampelos pareira* L. | Menispermaceae | 56.36 | 111.90 |
| *Aloe vera* (L.) | Asphodelaceae | 57.58 | 88.10 |
| *Micromeria biflora* Buch. | Lamiaceae | 57.58 | 90.48 |
| *Anisomeles indica* (L.) | Lamiaceae | 58.18 | 111.90 |
| *Pentanema divaricatum* Cass. | Asteraceae | 59.48 | 73.06 |
| *Helianthus annuus* L. (Sepals) | Asteraceae | 61.76 | 105.83 |
| *Lythrum salicaria* L. | Lythraceae | 62.86 | 148.87 |
| *Dichanthium annulatum* Forssk. | Poaceae | 67.41 | 89.04 |
| *Salvia moocroftiana* Wall. | Lamiaceae | 68.42 | 86.09 |
| *Phalaris minor* Retz. | Poaceae | 69.92 | 101.99 |
| *Cyperus rotundus* L. | Cyperaceae | 71.84 | 99.25 |
| *Saxifraga rotundifolia* L. | Saxifragaceae | 74.29 | 99.25 |

Results are significant at $P < 0.05$

TABLE 2
Evaluation of allelopathic activity by leaf litter of 73 medicinal weeds species through Dish pack method at the litter concentration of 100 mg

| Species | Extension % | | Criteria |
|---|---|---|---|
| | Radicle | Hypocotyl | |
| *Coronopus didymus* (L.) | 1.93 | 7.48 | **** |
| *Melilotus alba* Desr. | 6.77 | 30.73 | **** |
| *Melilotus indicus* L. | 9.67 | 41.53 | **** |
| *Malva parviflora* L. | 10.81 | 49.85 | *** |
| *Solanum nigrum* L. | 13.06 | 61.58 | *** |
| *Nasturtium officinale* W.T. | 22.14 | 66.05 | ** |
| *Urtica dioica* L. | 25.68 | 83.58 | ** |
| *Cyperus* sp. | 26.58 | 71.85 | ** |

TABLE 2 (Continued)

| Species | Extension % | | Criteria |
|---|---|---|---|
| | Radicle | Hypocotyl | |
| *Vicia sativa* L. | 31.98 | 79.91 | * |
| *Medicago parviflora* E.H.L. | 39.19 | 83.58 | * |
| *Achyranthes aspera* L. | 49.10 | 87.98 | |
| *Crotalaria medicaginea* Lamk. | 49.90 | 89.69 | |
| *Amaranthus viridis* L. | 52.71 | 113.79 | |
| *Xanthium strumarium* L. | 52.95 | 113.71 | |
| *Phalaris minor* Retz. | 53.68 | 70.60 | |
| *Avena fatua* L. | 57.54 | 93.02 | |
| *Oxalis corymbosa* DC. | 59.03 | 114.55 | |
| *Pentanema divaricatum* Cass. | 61.68 | 92.51 | |
| *Trichodesma indicum* (L.) | 65.76 | 107.14 | |
| *Plantago lanceolata* L. | 68.50 | 66.31 | |
| *Ipomoea cornea* Mart. | 68.58 | 115.38 | |
| *Lantana camara* L. | 69.37 | 122.43 | |
| *Salvia moocroftiana* Wall. | 70.60 | 81.40 | |
| *Cirsium arvense* (L.) | 71.83 | 85.71 | |
| *Cyperus rotundus* L. | 71.91 | 100.47 | |
| *Barleria cristata* L. | 77.68 | 95.57 | |
| *Helianthus annuus* L. (Sepals) | 77.68 | 85.63 | |
| *Cannabis sativa* L. | 78.34 | 117.11 | |
| *Phalaris aquatica* L. | 81.72 | 127.91 | |
| *Anagallis arvensis* L. | 82.69 | 122.92 | |
| *Conyza bonariensis* (L.) | 82.69 | 99.67 | |
| *Taverniera cuneifolia* Roth. | 83.64 | 99.42 | |
| *Sida cordata* (Burm.f.) | 84.27 | 105.50 | |
| *Centaurea iberica* T. | 86.81 | 124.58 | |
| *Convolvulus arvensis* L. | 87.50 | 149.48 | |
| *Saussurea heteromalla* D.Don. | 89.14 | 114.98 | |
| *Parthenium hysterophorus* L. | 89.93 | 116.74 | |
| *Carthamus oxyacantha* M. | 91.14 | 122.82 | |
| *Coriandrum sativum* L. | 92.13 | 108.89 | |
| *Digera muricata* (L.) | 93.69 | 100.77 | |
| *Chenopodium ambrosioides* Briq. | 93.81 | 110.47 | |
| *Taraxacum officinale* L. | 94.14 | 85.04 | |
| *Commelina benghalensis* L. | 94.85 | 98.32 | |
| *Micromeria biflora* Buch. | 95.21 | 90.00 | |
| *Dichanthium annulatum* Forssk. | 95.31 | 100.96 | |
| *Artemisia scoparia* W & K. | 95.72 | 100.00 | |
| *Pteris cretica* L. | 95.72 | 94.62 | |
| *Nerium oleander* L. | 96.74 | 109.23 | |
| *Scrophularia altaica* Murray. | 96.99 | 105.50 | |
| *Helianthus annuus* L. (petals) | 97.93 | 95.57 | |
| *Rhynchosia minima* (L.) | 98.15 | 105.05 | |
| *Sonchus asper* (L.) | 98.65 | 93.84 | |
| *Argyrolobium roseum* Camb. | 99.29 | 100.00 | |
| *Verbena tenuisecta* Briq. | 100.10 | 102.99 | |

TABLE 2 (Continued)

| Species | Extension % | | Criteria |
|---|---|---|---|
| | Radicle | Hypocotyl | |
| *Saxifraga rotundifolia* L. | 100.80 | 89.69 | |
| *Salvia aegyptiaca* L. | 101.83 | 106.92 | |
| *Otostegia limbata* Benth. | 102.46 | 89.18 | |
| *Sida alba* L. | 102.63 | 128.21 | |
| *Peganum harmala* L. | 103.59 | 108.01 | |
| *Oxalis corniculata* L. | 104.13 | 93.75 | |
| *Euphorbia hirta* L. | 104.99 | 100.92 | |
| *Rumex nepalensis* Spreng. | 106.31 | 118.04 | |
| *Adiantum caperis -veneris* L. | 106.42 | 102.31 | |
| *Typha minima* Funck. | 107.14 | 87.65 | |
| *Oenothera rosea* L' Her. | 111.22 | 99.67 | |
| *Solanum erianthum* D Don | 112.61 | 120.23 | |
| *Potamogeton lucens* L. | 117.13 | 105.61 | |
| *Lythrum salicaria* L. | 117.61 | 95.92 | |
| *Cissampelos pareira* L. | 117.65 | 105.08 | |
| *Aloe vera* (L.) | 118.53 | 109.76 | |
| *Anisomeles indica* (L.) | 121.19 | 92.99 | |
| *Euphorbia helioscopia* L. | 233.48 | 102.47 | |
| *Vernonia anthelmintica* L. | 234.24 | 115.81 | |

TABLE 3
Extraction of phytochemical contents of *Melilotous indicus*

| | TP content (µg GAE/ mg extract) | TF content (µg QE/ mg extract) |
|---|---|---|
| Methanolic Extract of *M. indicus* | 211.465517241379 | 190.694 |

TF= Total flavonoid, TP= Total phenolic.

of *Melilotus indicus*. On the basis of standard regression lines for gallic acid (y = 0.0232x - 0.0724; $R^2$ = 0.9991) and quercetin (y = 0.0252x + 0.0358; $R^2$ = 0.9971), the equivalents of TPC and TFC were calculated (Table 3). *M. indices* showed the maximum quantity of TPC (211.465517241379 µg GAE/g dry sample). Flavonoids were found to be rich in *M. indicus* 190.694 µg QE/ mg extract dry sample). The results of the phytochemical analysis of methanolic extracts of *M. indicus* are listed in table 4 (Table 4). Qualitative analysis of *M. indicus* ensured the presence of tannins, phenols,

TABLE 4
Phytochemical analysis of *M. indicus*

| | Alkaloids | Anthraquinones | Betacyanin | Coumarins | Flavonoids | Phenols | Saponins | Tannins | Terpenoids |
|---|---|---|---|---|---|---|---|---|---|
| *M. indicus* | ++ | - | ++ | + | +++ | +++ | + | ++ | + |

(+) present, (-) absent, (++) moderate concentration, (+++) abundant concentration.

flavonoids, and coumarins in plant methanolic extracts except anthraquinones were absent.

## DISCUSSION

Extracts from *M. indicus* show fairly good antibacterial and antitumor activities in screening experimentations (Miri, Rad, Alfatemi, & Rad, 2013). The occurrence of C-glycosides, methylene-dioxypterocarpan, pterocarpans, prenylated pterocarpan and flavone glycoside from this plant have been reported (Yadava & Jain, 2005; Ali, Shinwari, & Khan, 2019). Some literature reported that it has allelopathic potential to suppress seedling growth and germination (Gomaa et al., 2014). Previously no allelopathic activities or other ecological information was reported about the second strongly inhibitory *Medicago parviflora* species. *Melilotus alba* is found to be the third most inhibitory on lettuce seedling growth. Methanolic extracts of *M. alba* have best antitumor activities. Biochemical compound flavones, volatile oils, resins, and tannins are reported from *M. alba* (Stefanović, Tešić, & Čomićet, 2015). However, the allelopathic potential of *M. alba* has not been previously reported. *M. alba* in combination with other plants have been used in weeds management.

*Coronopus didymus* is found topmost strong allelopathic plants during Dish pack analysis. *C. didymus* commonly known as lesser swinecress native to South America particularly to Brazil is an annual but for some time biannual herb. *C. didymus* mostly found in disturbing situations such as cultivated land, roadsides, wasteland, winter farming, and uncompetitive pastures. The plant is valued in traditional medicine as a treatment for cancer, gangrene, hemorrhoids, allergies, and wounds. A plant decoction and leaf bandage are used to treat headache and fevers (Khaliq, Hussain, Matloob, Wahid, & Aslamet, 2013). Extracts of *C. didymus* has an allelopathic effect on wheat germination and its early seedling growth. Also applied to weed management assessment and extracts of *C. didymus* have allelopathic potential against seed germination of different plants (Marinov-Serafimov, 2010).

The plant allelochemicals have altered interactions among organisms, community dynamics into the soil environment and determine the development of plants in the soil (Meiners, Phipps, Pendergast, Canam, & Carsonet, 2017). Meanwhile, they affect the cellular structure, metabolism, photosynthesis, enzyme activity, nutrient absorption and hormonal regulation of target plant (Rehman et al., 2019). The extraction of allelochemicals from selective plant species using aqueous or alcoholic solvent comprehends the elaboration of allelopathic effects (de Moraes Gomes et al., 2017). Kasarkar and Barge (2016) applied the aqueous extract of *Azadirachta indica* leaves on wheat, mung, jowar, and cowpea and observed significant allelopathic effect on shoot length, biomass, root, and germination stage. Anwar, Khalid, Panni, Qureshi, and Rashid (2017) noted the suppression of germination and subsequent growth of *A. fatua, H. annus, Zea mays* and *R. dentatus* by aqueous extract of *Euphorbia helioscopia*.

Versatile compounds are present in plants which play an important role in pharmaceutical industries for the treatment of various complications and ailments in human as well in animals and plants. The presence of multipurpose phytochemical in plants gets more attention of researchers and physicians these days to deal with the increasing ratio of diseases (Petrovska, 2012). Plants are opulent by having various bio-compounds with diverse polarities (Jones & Kinghorn, 2006). Methanolic extracts have a large amount of TPC and TFC followed the rule that more compounds are solvable in polar solvents at room temperature (Pin et al., 2010). In present qualitative study of phytochemicals gives evidence of medicinal propensity of the selected weed. The allelochemicals were screened that impart biologically dynamic nature to the plants. The results of this screening analysis confirmed the presence of variant compounds terpenoids, coumarins, flavonoids, tannins, phenols, alkaloids, saponins, and betacyanin. These biologically

active allelochemicals have antioxidant, antimicrobial, anticancer, antifungal, and antidiabetic medicinal activities. Anti-inflammatory and hypoglycemic activities are reported from Tannins, flavonoids, and saponins while central nervous system activities and analgesic properties with terpenoids (Ullah, Jan, Gul, Khan, & Sher, 2018).

Treatment of various diseases with natural antioxidants get a worth reputation in this modern era and the traditional herbal medicinal value have been raised. During the assessment of the antioxidant capability of crude plant extract, the aptitude of DPPH radicle scavenging is considered as a milestone. This bioassay evaluation of plants antioxidants capabilities is very low-cost and time-limited (Majid et al., 2015). In this study, *M. indicus* showed enormous scavenging activity. Good scavenging capabilities may be exhibited by phenolics and flavonoids present in the huge amount due to the donation of electron or hydrogen to stabilize DPPH free radicles. Ullah et al. (2018) reported the antioxidant, analgesic, antimicrobial, anti-angionic, cytostatic and anti-allergic activities of flavonoids. Therefore these observations support the utilization of *M. indicus* in herbal medications and recommended as a potential source of significant allelochemicals for the pharmaceutical industry.

In view of results obtained from quantitative and qualitative analysis various compound have been identified from *M. indicus* methanolic extracts of leaves. The main ingredients present in methanolic extracts were total phenolic content and possible high fungicidal activity is due to the presence of phenolic components. TPC compounds are abundantly found plants secondary metabolites and have about ten thousand individual compounds (Majid et al., 2015). Phenolic or polyphenol can be defined chemically as a substance which possesses a benzene ring with one or more hydroxyl groups, with evidence that increased hydroxylation results in increased toxicity. Plants showed some resistance to microorganism, herbivores and insects are mainly due to these polyphenols. The obtained results of this study were agreed with previously stated that the resistance to phytopathogenic microorganisms is because of the phenolic pathway activation (El-Khateeb et al., 2013). Sisti et al., (2008) also stated the TPC are vigorous to counter microorganisms of pathogenic nature for human beings and animals. Phenolic components counteract envelopment of harmful mediators through several proposed modes of action. These involve in enzymatic processes impairing, weakening the synthesis structural element, rescinding cell membrane permeability barrier, changing the cells physiological eminence and affecting the synthesis of nucleic acids (Bajpai, Sharma, & Baekt, 2013; Fadli et al., 2012).

The noxious weeds with medicinal properties may have strong allelochemicals potential that can help in the development of bioactive compounds from plant species to be used as natural herbicides for environmentally friendly sustainable control of weeds. *M. indicus* was identified as the strongest allelopathic plant. The result showed that *M. indicus* strongly inhibit the seedling growth of test weed species. Various allelochemicals i.e. TPC, TFC, tannins, phenols, flavonoids and coumarins were identified from the extract of *M. indicus*. There is a dire need to develop a complete database of plants having strong allelopathic potential. It is also recommended to isolate and enlist the allelochemicals present in weeds that's may be used for weed and pest control.

**Ethical statement:** authors declare that they all agree with this publication and made significant contributions; that there is no conflict of interest of any kind; and that we followed all pertinent ethical and legal procedures and requirements. All financial sources are fully and clearly stated in the acknowledgements section. A signed document has been filed in the journal archives.

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## RESUMEN

**Potencial alelopático de 73 especies de malezas en Pakistán. Introducción:** El uso de aleloquímicos como sustitutos baratos y amigables con el ambiente para el manejo de hierbas en plantaciones es un tema central actualmente. **Objetivo:** En el presente estudio se midió la actividad alelopática de 73 hierbas en Pakistán a través de los métodos "sandwich" y "dish pack", con el fin de investigar los compuestos aleloquímicos más importantes en las hierbas analizadas. **Métodos:** A través del método "sandwich" se determinaron los efectos alelopáticos de los lixiviados de hojas en las hierbas analizadas, mientras que el efecto alelopático volátil a través de la técnica "dish pack". Para investigar el potencial aleloquímico de *Melilotus indicus* se aplicaron técnicas fitoquímicas tanto cualitativas como cuantitativas. El análisis estadístico de los datos describió el efecto alelopático de 73 especies de hierbas sobre el crecimiento radicular y del hipocótilo en plántulas de lechuga. **Resultados:** El porcentaje de elongación radicular y del hipocotilo varió entre 0-74 % hasta 0-148 % y 0-75 % hasta 0-84 % respectivamente, con una concentración de 10 mg de polvo de planta seca con el método "sandwich". Además, fue entre 2-234 % y 7-150 % con el método "dish pack". Entre las plantas analizadas para actividad fitotóxica, *M. indicus* presentó los aleloquímicos más fuertes. Los análisis cualitativos y cuantitativos de *M. indicus* mostraron la presencia de flavonoides y compuestos fenólicos, junto con otros aleloquímicos. **Conclusiones:** Las hierbas seleccionadas pueden tener un potencial aleloquímico fuerte que ayude al desarrollo de compuestos bioactivos de plantas, para usar como hierbicidas y pesticidas naturales en el manejo sostenible de plagas y malas hierbas.

**Palabras clave:** alelopatía; bioensayo; ELISA tipo sándwich; fitoquímicos.

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