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IN VITRO SCREENING OF *CORONOPUS DIDYMUS* AND *NASTURTIUM OFFICINALE* FOR WEED AND PEST CONTROL IN AGRO-ECOSYSTEMS

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Abstract - Allelopathy is a viable alternative in agro-ecosystems for weed and pest control. The present work was aimed at evaluating the herbicide and pesticide ability of *Coronopus didymus* (L.) Sm. and *Nasturtium officinale* W.T. Aiton The allelopathic effect of selected weeds was calculated by sandwich method and dish-pack bioassay. Aqueous extracts of both plants were also used to determine the effects on crop growth in seedlings (wheat and maize). Leachates of *C. didymus* and *N. officinale* had an inhibitory effect on the growth of lettuce seedlings by 98.8% and 98.5% respectively in dish pack bioassay; lettuce seedling radicle elongation inhibition was 1.9% and 22.1% respectively compared with untreated control. The inhibition effect for wheat and maize was of no importance. Micro-spectrophotometric technique was used for fungicidal assessment. Both plant extracts (3.125 mg/mL) demonstrated substantial pesticide activity against pathogenic strains *Pyricularia oryzae* Cavara and *Fusarium fujikuroi* Nirenberg. *C. didymus* reported maximum total phenolic content (214.6983 µg GAE/g dry sample) *and N. officinale* (124.181 µg GAE/g dry sample). Identification of new active compounds can aid in weed and pest management.

Keywords: crop production; allelopathy in agro-ecosystems; sandwich method and dish pack bioassay; micro-spectrophotometric technique; total phenolic content.

Introduction

The projected annual rise of the world population by 30 percent would lead to 9.2 billion masses by 2050. Massive population growth coupled with changing nations' eating habits to get high-quality food has led to food insecurity. The trend will soon lead to a 70 per cent rise in food production demand (Popp et al., 2013). At the other side, the agricultural land will be expanded to meet increasing food requirements and demand, which would lead to deforestation and natural habitat destruction. Therefore, by using modern and technological practices, production with available land, energy, water and other resources is urgently needed (Zhang et al., 2017; Vongvisouk et al., 2016). Specific effectiveness risks include inadequate crop feed, infections, weeds, pests (micro-organisms and insects) and abiotic stress. Climate change is another major challenge to food sustenance. The crop yields were significantly reduced by increasing temperatures and evolving patterns of precipitation (McDonald et al., 2009; Semenov & Halford, 2009; Farooq et al., 2013).
Worldwide, weeds that cost more than $18.2 billion in agronomic output loss are reducing 10 per cent of annual agricultural

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production (Kadioglu et al., 2005). Weed management and crop protection is an emerging concern in modern farming practices as the growing use of pesticides has adverse effects on both health and the environment (Petrova et al., 2015). Theoretically, the solution to the issue is the creation of new technologies, processes, strategies and biodegradable chemicals that have less harmful impacts, for weed and pest control. Production of large and cash crops is mostly at risk of pathogenic pest attack causing loss of yields. The pest attack triggers a yield loss of about 50 per cent and 80 per cent in wheat and cotton respectively worldwide. Savary et al. (2017) announced an estimated cotton yield loss of 37%, wheat 31%, maize 40%, and soybean 26-29%. The risk of infectious diseases of plants has risen in both natural and agricultural environments over the last two decades. Noxious and phytopathogenic species play a critical role in the loss of crop production and biodiversity of the forests like large trees (Fisher et al., 2012). Fungal diseases such as collar rot stem rot, root rot and so on damage commercial plants (Khan et al., 2017).

The crops are protected from insect attack and infestation of weeds by the application of various pesticides and advanced biotechnological techniques and products in general practice. If crop protection techniques and procedures are not adopted, then food insecurity in the world can result (Vikkey et al., 2017). Exposure to these pesticides caused numerous health problems in children such as brain cancer and leukemia, and high miscarriages have been reported in adult ladies (Hertz-Picciotto et al., 2005). Pesticides cause acquired heart defects and can also affect the nerves and respiratory systems (Rallis et al., 2014). The use of less costly, effective and environmentally friendly weed and pest control technologies to achieve sustainable growth and optimize production has gained more attention in agro-science research, especially weeds allelopathy (Om et al., 2002). Using old farming methods such as reseeding, seeding, covering crops and crop rotation has the potential to lead to better results along with the use of allelopathic action (Chon et al., 2006). Over time the resistance of weeds to synthetic herbicides increases. Therefore, plants with herbicide potential that could supply phytochemicals are considered a significant benefit for the pesticide industry (Kruse et al., 2000; Bhowmik, 2003).

Allelochemicals have substantial plant protection capacity, crop growth and biological control. Allelochemical features that make them potentially 'natural herbicides' are: similar mode of action to synthetic herbicides, partial/total water solubility without surfactants for easy application, environmentally safe chemical structures with higher oxygen and nitrogen content and non-halogenated molecules that minimize the half-life of the atmosphere while preventing accumulation of oxygen and nitrogen content. Examples of allelochemicals playing a role in weed control are phenolics and isoflavonoids, allyl isothiocyanate, scopoletins, hydroxamic acids (Qureshi et al., 2019).

Coronopus didymus (L.) Sm. and *Nasturtium officinale* W.T. Aiton are well-recognized allelopathic plants. Both species are members of family Brassicaceae and grow profusely in cultivated as well abandoned fields, lawns, waste ground and vacant plots (Khaliq et al., 2013). Little information is available on the possible allelopathic interference of these plant species on weeds. The present research work has therefore been planned to investigate the phytotoxic effects of aqueous and methanol extracts of *C. didymus* and *N. officinale* on germination dynamics and seedling growth of weed seedlings as well as the pesticide potential against selected phytopathogenic strains.

Materials and Methods

Collection of Plant Materials

The fresh leaf liters of *Coronopus didymus* (L.) Sm. and *Nasturtium officinale* W.T. Aiton were collected and put at 45°C in a drying oven. For more experimental work the dried material was packed separately.

Allelopathic Screening of Selected Weeds

Sandwich Method: Methodology described by Fujii et al. (2004) was adopted. Agar is recommended as a support medium for germinating seeds and growing seedlings. The agar (7.5g/1000 mL of distilled water) was used as a support medium in the current research. The solution was prepared in autoclave at 115°C for 15 minutes. Previous work recommended *Lactuca sativa* seeds which are extremely phytochemically sensitive and the most trustworthy for germination analysis (Appiah et al., 2015). Phytotoxicity was therefore verified on lettuce seeds. Five seeds were placed in each petri plate providing an agar medium of growing test species. Experiments were performed in triplicate at 10 mg and 50 mg of dried plant material. All the plates were coated with plastic tape (120 plates for 10 test weeds) and wrapped in aluminum foil. The plates were incubated at 25°C for 3 days. The control was set up without plant material. Measurements of the seedling growth were taken with graph paper and tweezers.

Dish Pack Method: Following Fujii et al. (2005), the dish pack technique was used for study of allelopathic effects. For this purposes the six-well plates were used. The distance from the source well for each well of multi-well plates was 41, 58, 82, and 92 mm (Figure 1). 100 mg of dried leaves were placed in the source well, while in others filter paper

was placed and distilled water (7 mL) was applied to each well. With the exception of source well, five lettuce seeds were put in each well. The multi-well plate was sealed and incubated for 3 days at 25°C. Measurements of the seedling growth were taken with graph paper and tweezers.

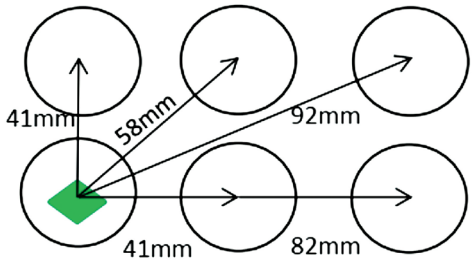

Figure 1. Model for analysis of allelopathic activity by dish-pack method.

Assessment of Allelopathic Potential on Crops

Extract Preparation

Using a milling machine, the oven dried leaves were grinded to powder. Aqueous extract of *C. didymus* and *N. officinale* was prepared by combining distilled water (1000 mL) with dry powdered plant biomass (100 g) at 25°C, for 24 hours (Norsworthy, 2003). Muslin cloth and filter paper were used for filtration. The final 10 per cent (100 g/L) concentration was prepared by dilution with distilled water.

C. didymus and *N. officinale* methanol extracts were prepared by mixing 100 g of powdered plant with 500 mL of methanol twice at room temperature for 48 hours (Basri & Fan, 2005). After filtration, the extracts were concentrated on reduced pressure at 40°C using a rotary evaporator. The extracts were reconstituted at a concentration of 50 mg/mL in dimethyl sulfoxide (DMSO) for pesticide activity evaluation. The absence of turbidity in the broth after the incubation period indicated a sterile extract. The extracts were stored at 4°C for further use.

Effect on germination and growth of crops

Wheat and maize seeds were obtained from the National Research Center for Agriculture (NARC). The filter papers were put in sterilized petri dishes. 5 seeds (sterilized with 0.1 % $HgCl_2$) were put in Petri dish and 5 mL of 10% aqueous extract was applied directly to wheat seeds. Experiment was performed in triplicate. Distilled water was used as control. Petri plates were incubated at 25°C. Growth measurements including germination rate, shoot length and root length were taken after 7 days (Kasarkar & Barge, 2016). In the second part of experiment, 5 seeds of wheat were planted with soil with same setup. The dishes were watered with aqueous extract of each selected plant, while the control was watered with distilled water. Hypocotyl and the radicle length of the seeds were calculated after 7 days and compared with the control. The same experiments were performed with maize seeds.

Pest Control Technique

For assessment of pesticide potential of *C. didymus* and *N. officinale* against *Fusarium fujikuroi, Alternaria alternata, Macrophomina phaseolina, Fusarium oxysporum, Rhizoctonia oryzae, Rhizoctonia solani, Pyricularia oryzae* and *Pythium ultimum* strains, the Sabaruad dextrose agar medium was used for the growth of selected strains at 25°C for 48 hours. The suspension was then made at 630 nm with an optical density (OD) of 0.1.

Pesticide activity assessment was done following micro-spectrophotometric automated quantitative method (Broekaert et al., 1990). Measurement of growth inhibition in 96-well microtiter plates was carried out at 630 nm. Protocol for extract evaluation using 10µL spore suspension, 20µL of extract prepared by adding 50 mg/mL in DMSO, and 70µL potato dextrose broth (PDB) was adopted. A negative control was set with 20µL of DMSO while 0.2 mg/mL 'Nystatin' was used as positive control.

For the determination of extract MIC values microplate system with minor adjustments was used (Khan et al., 2018). This process integrates the sequential dilution of plant extract from crude concentrate (1/2 to 1/100 dilutions). Each microplate well contained pathogen spore suspension (100 µL) against each 100 µL dilution. The microplates were incubated in triplicates at 25°C for two days. The evaluation was then performed with microplate reader via spectrophotometer at 630 nm. The MIC values were suggested by the association between production in control wells and clear concentrate in not-inoculated plates. For absorbance measurement of spore containing plates at 630 nm, an ELISA plate reader technique was applied. The pathogen growth was measured in triplicate after two days of incubation at 27°C. The following equation was used for determination of growth inhibition,

$$\text{Growth Inhibition} = [(\Delta C - \Delta T) \div \Delta C] \times 100$$

Where ΔC = control micro-culture corrected absorbance at 630 nm; ΔT = tested micro-culture corrected absorbance.

Phytochemical Analysis of Methanolic Extracts

The overall phenolic content was measured by the use of a spectrophotometer (Kim et al., 2003). The sample was prepared in a volumetric bottle (25 mL) by adding 1 mL of plant material in 9 mL of deionized water followed by adding 1 mL of Folin and Ciocalteu's phenol reagent, adding 10 mL of 7 per cent of sodium carbonate and pure deionized water after 5 minutes of vigorous shaking until the final volume was 25 mL. Solution was held at 23°C for 90 minutes after stirring. The optical density was calculated at 750 nm. Gallic acid at concentration range of 0-2 mg/mL), dissolved in ethanol/water (75:25, v/v, 0.3% HCl) was used as the standard.

The spectrophotometric technique was used for quantifying the total content of flavonoids (Park et al., 2008). A test tube containing 0.3 mL of plant extract (1mg/mL; 0.1 percent), 0.15 mL of 0.5 mol/L $NaNO_2$, 0.15 mL of 0.3 M $AlCl_3 6H_2O$ and 3.4 mL of 30 percent aqueous methanol was used to prepare the reaction mixture. 1 mL of NaOH was added after 5 minutes, and optical density was measured at 506 nm using 0.10 mg/mL'rutin' solubilized with ethanol 50% (v/v) as the standard.

Statistical analysis

The data obtained by sandwich method and dish pack assay at 10 and 50 mg concentration of plant materials, wheat and maize seedling growth inhibition by aqueous extract of *Coronopus didymus* and *Nasturtium officinale* on filter paper and soil and percentage inhibition of pathogenic strains was subjected to Analysis of variance (ANOVA) followed by Student-Tuckey test in SPSS v.21. Statistical significant difference among values of assays is indicated by different letters (a,b,ab,c,d,cd…) on the top of bars in graph.

Results and Discussion

Weeds are unwanted species of plants that can grow in agricultural fields with different bodies and have negative impacts on the production and yield of final crops (Bhowmik & Doll, 1992; Marwat & Hashim, 2002). Cash crops such as wheat, corn, sugar cane, and fruits face weedy flora infestation. In agro-ecosystems the uncontrolled weed issue significantly reduces crop production. Plants release different types of secondary metabolites into the atmosphere. Exploring and researching these bio-compounds can lead to their beneficial uses, as well as avoiding negative synthetic impacts. However, on the basis of classification of phytochemicals and their impacts on the natural and agricultural environment and also their use for biological control of weeds and pests, there is no comprehensive research and literature available. In current study, *Coronopus didymus* (L.) Sm. and *Nasturtium officinale* W.T. Aiton have been inhibitory in germination and seedling growth of weeds, indicating a potential allelopathic intervention under field conditions.

For the assessment of allelopathic ability, the sandwich method and dish pack bioassay were carried out. The findings of bioassay showed that *C. didymus* and *N. officinale* have inhibitory effect on lettuce seed growth (Figure 2a). At 10 mg of dried leaves, *C. didymus* and *N. officinale* inhibit 63.15 and 84.21 percent radicle elongation and 88.39 and 96.42 percent hypocotyl elongation. 50 mg of *C. didymus* and *N. officinale* showed 92.98% and 84.21% radical inhibition, and 98.21% and 96.42% hypocotyl inhibition, respectively. Results of the dish pack method described allelopathic effects of *C. didymus* and *N. officinale* on lettuce seedling elongation (Figure 2b). Radicle elongation was 1.9 percent, and 22.1 percent, respectively for *C. didymus* and *N. officinale* as compared to control.

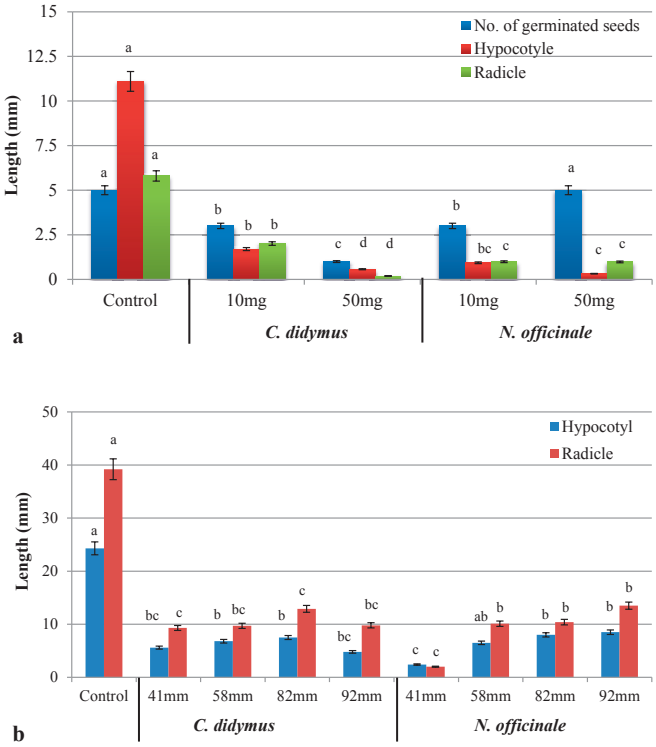

Figure 2. Lettuce seedling growth inhibition by *Coronopus didymus* and *Nasturtium officinale* (a): Sandwich method at 10 & 50 mg plant material (b): Dish-Pack method at 100 mg of plant material.

The findings showed a minimum hypocotyl growth of 1.5 mm and a maximum length of 16 mm by applying *C. didymus* aqueous extract on wheat. In the case of *C. didymus* aqueous extract in wheat the radicle length ranged from 1.5-7 mm. The minimum length of hypocotyl was 3.5 mm, and the maximum length of 21 mm was recorded during wheat exposure to aqueous *N. officinale* extract. On the other hand, a minimum radicle length of 4.5 mm was reported, and a maximum of 21.5 mm. The maize seeds had been reacting differently to aqueous extracts. Exposure by aqueous extract from *C. didymus* resulted in hypocotyl germination of seed ranges from 2.5-9 mm and 1.5-12 mm. The maximum hypocotyl length was reported as 16 mm in *N. officinale* aqueous extract whereas the radicle length was 14 mm in *N. officinale* aqueous extract. In wheat seeds the radicle and hypocotyl length ranged from 9.5-22 mm and 10.5-24 mm respectively in soil treated with

aqueous *C. didymus* extract. In the soil containing aqueous extract of *N. officinale*, maize hypocotyl length ranged as 15-29 mm and radicle length was 19-30 mm. Seed hypocotyl length ranged from 10-13 mm and the length of the radicles varied from 14.5-27 mm. The aqueous extract of *N. officinale* strongly inhibited the germination of the seed in the soil, the length of the hypocotyl ranged from 15-24 mm and 11.5-39 mm of radicle length (Figure 3).

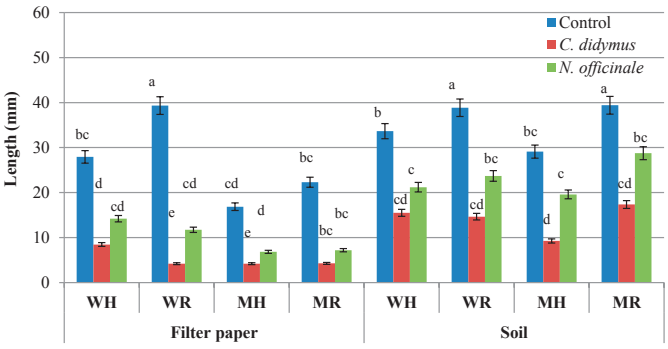

Figure 3. Wheat and Maize seedling growth inhibition by aqueous extract of *Coronopus didymus* and *Nasturtium officinale* on filter paper and soil (WH = Wheat hypocotyl; WR = Wheat seedling radicle; MH = Maize hypocotyl; MR = Maize radicle).

Weeds harbors the pathogenic pests that cause various diseases of plants and these diseases spread in both agricultural and natural habitats (Dangwal et al., 2010). Phyto-pathogenic strains cause a number of diseases, such as root rot, stem rot, crown rot, damping-off, vascular wilts, resulting in major economic losses in crop yield and quality worldwide. Using synthetic herbicides is the best way to tackle this issue, but there is a high risk of many direct and indirect related hazards to human health and environment (Ahmed et al., 2015). Secondary plant metabolites known as allelochemicals have low environmental impacts compared to synthetic chemicals and are the inspiration to produce natural products (El-Abbassi et al., 2017). Plant pesticide activity is previously recorded (Zahradníková & Petříková, 2012; Nikan & Khavari, 2014; Riaz et al., 2017). In our study, for each strain, the MIC value of *C. didymus* and *N. officinale* extracts varied. The MIC value of 12.5 mg/mL was against the *Pythium ultimum, Rhizoctonia solani, Fusarium oxysporum* and *Macrophomina phaseolina* (least susceptible strain) for both plants. *Pyricularia oryzae* and *Fusarium fujikuroi* demonstrated MIC of 0.781 mg/mL (most susceptible strain). The MIC value for 'Nystatin' ranges from 15.25-1250 U/mL (Table 1). Both extracts had minimum fungicidal concentration (MFC) values in the range of 3.125-25 mg/mL. Detected against *P. oryzae* and *F. fujikuroi* the minimum MFC value was 3.125 mg/mL. *F. oxysporum* and *M. oryzae* MFC values ranged from 12.5-25 mg/mL for *Pythium ultimum* (Figure 4; Table 1).

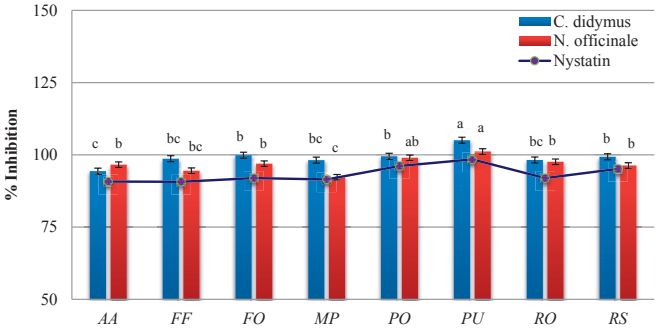

Figure 4. Percentage inhibition of pathogenic strains by 5% extract of *Coronopus didymus* and *Nasturtium officinale* in Dimethyl sulfoxide. PU = *Pythium ultimum*; RS = *Rhizoctonia solani*; PO = *Pyricularia oryzae*; FF = *Fusarium fujikuroi*; RO = *Rhizoctonia oryzae*; FO = *Fusarium oxysporum*; AA = *Alternaria alternate*; MP = *Macrophomina phaseolina.*

Table 1. Minimum inhibitory concentration (MIC) and Minimum fungicidal concentration (MFC) of crude extracts of *Coronopus didymus* and *Nasturtium officinale*.

		PU	RS	PO	FF	RO	FO	AA	MP
C. didymus	MIC*	12.5^{a}	12.5^{a}	0.78^{d}	0.78^{d}	3.13^{c}	12.5^{a}	6.25^{b}	12.5^{a}
	MFC*	12.5^{b}	12.5^{b}	3.125^{d}	3.125^{d}	6.25^{c}	25^{a}	6.25^{c}	12.5^{b}
N. officinale	MIC	12.5^{a}	12.5^{a}	0.78^{d}	0.78^{d}	6.25^{b}	12.5^{a}	3.125^{c}	12.5^{a}
	MFC	25^{a}	12.5^{b}	3.13^{d}	3.13^{d}	12.23^{b}	12.5^{b}	6.25^{c}	12.5^{b}
Nystatin**	MIC	1250	1250	156.3	156.3	625	1250	1250	1250
	MFC	2500	2500	312.5	312.5	1250	2500	1250	2500

*MIC and MFC are presented in mg/mL; ** Nystatin was used in units/ml.
Different letters as superscripts in table indicate statistical significant difference among values
PU = *Pythium ultimum;* RS = *Rhizoctonia solani;* PO = *Pyricularia oryzae;* FF = *Fusarium fujikuroi;* RO = *Rhizoctonia oryzae;* FO = *Fusarium oxysporum;* AA = *Alternaria alternate;* MP = *Macrophomina phaseolina.*

Total phenolic and flavonoid content were identified from methanolic extract *C. didymus* and *N. officinale*. On the basis of standard regression lines for gallic acid (y = 0.0232x - 0.0724; R^2 = 0.9991) and quercitin (y = 0.0252x + 0.0358; R^2 = 0.9971), the equivalents of TPC and TFC were calculated. *C. didymus* showed maximum quantity of TPC (214.6983 µg GAE/g dry sample) and *N. officinale* (124.181 µg GAE/g dry sample). Flavonoids were found to be rich in *C. didymus* and *N. officinale* as 170.8532 µg QE/mg and 113.7103 µg QE/mg methanol extract respectively (Figure 5). The high phenolic content in the current study may lead to allelopathic properties (Szwed et al., 2019).

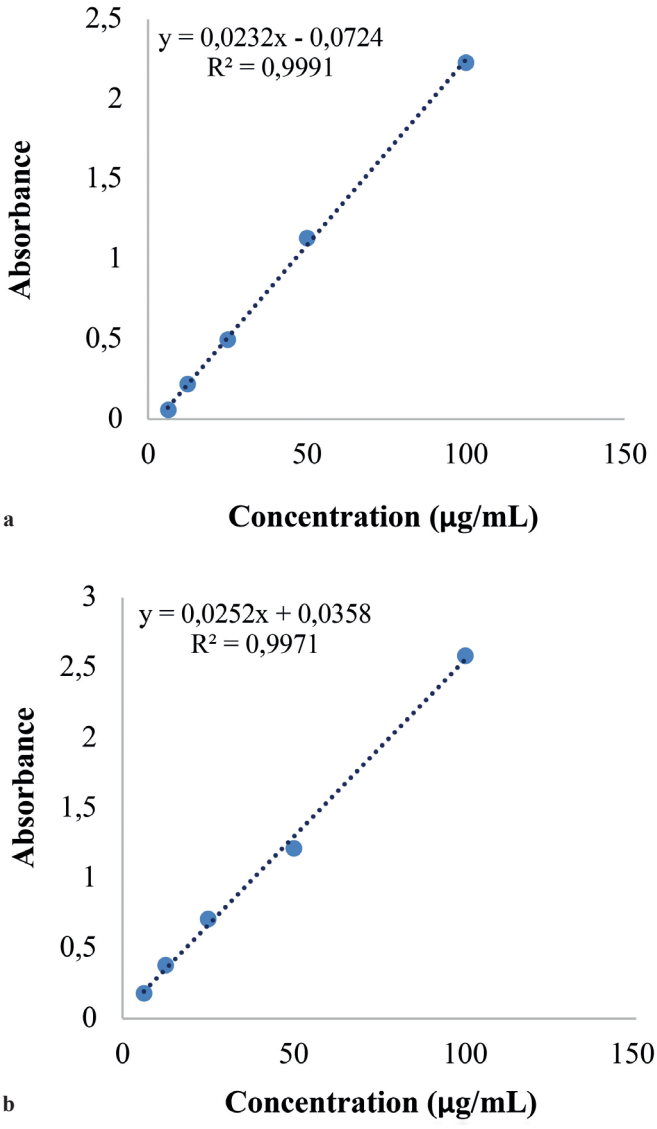

Figure 5. (a): Calibration curve of quercetin for total flavonoid determination; (b): Calibration curve of gallic acid for total flavonoid determination.

Conclusions

The use of less costly, effective, and environment friendly weed and pest control technologies has gained interest in research on agro sciences. Possibly the plants with herbicide potential will be considered for the pesticide industry. The current study provides evidence that *Coronopus didymus* and *Nasturtium officinale* have allelopathic potential. In both sandwich and dish pack assay, the two species had a strong allelopathic effect on the growth of lettuce seedlings. The extracts have demonstrated fungicidal activity on tested fungal strains. Selected weeds may also be used to grow eco-friendly natural pesticides that can be used against diseases that cause pathogens such as *R. solani, R. oryzae, F. fujikuroi, F. oxysporum, P. ultimum* and *P. oryzae* and cropping system weeds without harming the ecosystem.

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