Molecular Characterization and Allelopathic Potential of Radish Species on Wheat and Weed Species

Saman A. Rasul*, Kawa A. Ali

College of Agricultural Engineering Science, Salahaddin University-Erbil, Kirkuk Road Street, Erbil City.

*Corresponding author's Email: saman.rasul@su.edu.krd

Abstract. This study was conducted at Salahaddin University/ College of Agricultural Engineering Science, to inspect the allelopathic effects of radish (Raphanus sativus L.) on two wheat species and two endemic weed species. The study included two experiments, first one was bioassay experiment to estimate the aqeous extracts effect of three radish species (red, black and white) at five concentration levels (0, 10, 20, 30, and 40%) on germination and seedling growth of bread wheat (Triticum aestivum Var. Adana), hard wheat (Triticum durum Def. Var. Smito) and two endemic weed species wild oat (Avena fatua) and wild barely (Hordeum spontaneum). Results indicated that aqueous extracts of black radish possess more allelopathic potential compare to red and white radish, also, aqueous extracts significantly affected on all studied plant species at dissimilar ratio, while most effective concentration was (40%). Whereas the germination of all plant species totally inhibited at this concentration. The second experiment aimed to indicate the genetic relatedness (genetic diversity) among radish species by using Random amplification of polymorphic DNA (RAPD-PCR) technique. The results indicate that the genetic distance among the three studied radish species ranged from 17.33 to 37.6. The lowest genetic distance was recorded between black and red that was 17.33 and the highest genetic distance recorded between black, red with white was 37.6 and white radish with each of black and red radish were 65.067. the results suggested that radish species have different allelopathic potential and genetic variance exist among them.

1. Introduction

Allelopathy has been first introduced by [1] and identified as biochemical interactions between plants, including microorganisms. Therefore, allelopathy can be defined as a mutual harm or benefit between different plants through releasing compounds that enter the environment, allelochemicals produced naturally or as a result of mechanical and physiological damage in plant as secondary compounds stored in vacuole then released into environment [2]. Different plant species were found to have allelopathic activities which have a potential application in agro ecological systems [3]. Among these families, brassicaceae has had more interest from researchers [4] Radish from brassicaceae family has been found by previous studies to have allelopathic effect on different plants species [5,6], resulted from its secondary metabolite compounds such as p-hydroxy benzoic acid, and isoiothiocyanates (isothiocyanate benzyl, Isothiocyanateallyl) [7,8]. The allelopathic activity of brassicaceae family on weeds has been examined in different aspects. Some crops and weeds showed germination sensitivity to wild radish (Raphanus raphanistrum L) aqueous extract at various levels and radicle growth of some weeds was inhibited [9,10] Turk and Tawaha (2003) found that germination and seedling growth of wild oat (Avena fatua) was inhibited by aqueous extracts of black mustard (Brassica nigra). Some Brassica species, such as radish (Raphanus sativus var. niger), white radish, black little radish, rapeseed (Brassica napus var. oleifera) and turnip (Brassica campestris sub sp. rapa) were found in recent studies to be effective for controlling Johnson grass [11]. There are various species of radish around the world and these species differ from each other in size, shape, and color of root, also in root diameter, length, and weight [12]. The open pollination habit is one of the reasons that helped the species to accumulate a number variation. Further to the previous information, it was shown that the flora morphology exhibited significant influence among the radish accessions [13]. Taxonomy and genetic variance of radish germplasm is unclear and insufficient studies have compared and classified the phenotypic diversity of different species [12]. In addition, the variations provided large genetic resources in order to increase genetic of radish. Thus, the evaluation on the genetic diversity of radish can be the reason to make the utilization and improvement of radish germplasm. There are abundant number of molecular markers, for example random amplified polymorphic DNA (RAPD) [14], and amplified fragment length polymorphism (AFLP), have been applied to evaluate the genetic diversity of radish respectively. RAPDs are known as arbitrary primed Polymermerase Chain Reaction (AP-PCR), this technique is based on the use of short random primers in a PCR reaction and can be used to produce relatively detailed and complex DNA profiles for detecting amplified fragments between
organisms. For surveying the genetic variations of radish, morphological characteristics and RAPD markers were used [15,16]. The application of the random amplified polymorphic DNA technique [17,18] was successfully utilized in the cruciferous species for the purpose of cultivar identification and evaluation of genetic similarities and for diversity among germplasm of crops [19]. It has been revealed that, morphological characters and molecular markers (random amplified polymorphic DNA, RAPD), were conducted to evaluate the genetic diversity and the relationships among radish germplasm collected from some regions of Pakistan [15]. The main advantage of RAPDs technique is that they can achieve at a reasonable cost and will generally amplify a range of fragments of most DNA and show polymorphisms.

2. Materials and Methods

This study comprised two experiments to estimate the allelopathic potential of radish (Raphanus sativus L.). The bioassay experiment aimed to distinguish the allelopathic potential of three species of radish (red, black and white) on germination and some growth parameters of bread wheat (Triticum aestivum L.), hard wheat (Triticum durum Def.), wild barley (Hordeum spontaneum L.), and wild oat (Avena fatua L.). The second experiment, genomic DNA of radish was extracted and molecular analysis of radish genomic DNA was performed by using RAPD-PCR technique.

2.1. Seed Source

The seeds of radish species (Raphanus sativus L.), bread wheat Triticum aestivum var. Adana, hard wheat Triticum durum Def. Var. Smito, wild barley Hordeum spontaneum L., wild oat Avena fatua L., were received from Erbil Agricultural Research Center.

2.2. Sample Collection and Preparation of Aqueous Extract

Three commercial seeds of radish's genotypes (black, red and white) were sown in plots in Gdrarasha farm/ College of Agricultural Engineering Science/ Salahaddin University-Erbil in August, 2016. The root of radishes collected after 40 days of planting. The radish roots washed with tap water after that it was cut in to 4-5 cm pieces to be put in a blender to obtain radish aqueous extracts without adding any amount of water. Finally, it filtered by Withman filter paper No.1. The resultant aqueous extracts were stored at -20 °C until required for the bioassay and field experiments [20].

2.3. Bioassay

Bioassay process conducted by preparing five concentrations (0, 10, 20, 30 and 40%) of the previously stored radish root aqueous extract, and distilled water was added to reach wanted concentration, whereas sterilized water was used as control. Twenty seeds of each plant species were put in 9 cm Petri dish and then treated with 10 ml of the concentrations of radish aqueous extract. Parafilm tape was used to seal Petri dishes and placed in the incubator 20-22 o C. Germination percentage of each Petri dish was determined on 3rd, 5th, 7th and 10th day.

2.4. Recorded data

The recorded parameters were seed germination percentage, inhibition of germination %, seedling growth inhibition% [21] Radicle length (cm), plumules length (cm), plumules and radicle elongation velocity (cm/day), radicle, plumules dry weight (mg), seedling dry weight (mg), total dry weight (mg) and seedling vigor index [22]as detailed below:

Germination% = T.G.S./T.T.S.*100 …………………………………………. (1)
I.O.G. %= (C.G.P. – T.G. P)/ C.G.P 100 ……………………………. (2)
P.E.V. (cm/day) or R.E.V. (cm/day) = [(P.L. (cm) or R.L. (cm))/T.D.] *100 ……… (3)
S.V.I. ={S.L. (cm)*G %}/100…………………………………………… (4)
S.G.I. = G% *S.L. (cm/100) ……………………………………………………………… (5)

Where G% = germination Percentage, I.O.G.= inhibition of germination, T.G.S=total germinated seed, TTS = total tested seeds, C.G.P. = control germination percentage, T.P.G =treatment germination percentage, PEV= plumule elongation velocity, TD= total days, RL= radicle Length, PL= plumules length , SL= seedling length (cm), SVI= seedlings vigor index.

2.5. Total Genomic DNA Extraction
Genomic DNA extraction was performed at laboratories of Agricultural engineering science/ Salahaddin University. Ten fresh radish roots were randomly selected from each species. DNA isolation kit (Plant DNA Preparation Kit Jena Bioscience/ Germany). Based on the Kit 50 mg of fresh root was extracted and then followed the Kit instruction manual, then DNA samples stored at -20 °C until required. According to Plant DNA preparation Kit the procedure was as follow:

2.6. DNA Quantification and Qualification

The Spectrophotometer model Nanodrop1000 manufactured by Thermo Scientifics (Fig. 1) Designed for measuring nucleic acid concentrations in sample volumes of one micro liter. This accomplished by placing the sample directly on top of one detection surface and using the surface tension to create a column between the ends of optical fibers. Thus, the measurement optical path is formed. The sensitivity range for DNA detection is between 2 and 3700 µg/µl. The spectral range of the device is 220 to 750 nm and it is possible to scan all of the wavelengths. The instrument is driven by a computer, which allow 280nm. This ratio is used to assess the purity of DNA and RNA. A ratio of 1.8 is generally accepted as “pure” for DNA; a ratio of 2.0 is generally accepted as “pure” for RNA [23]. Finally, the quality of DNA was checked by agarose gel 1%. Following procedure used to determine the concentrations and purity of DNA samples by Nanodrop.

2.7. RAPD Primers

As shown in Table 1. Three primers were used in this study, and the primers purchased from (CinnaGen-Iran) company [24].

Table 1. Primer name, sequence and GC% for all primers.

| No. | Primer name | Primer sequence 5 to 3 | GC%  |
|-----|-------------|-------------------------|------|
| 1   | OPA-14      | TCTGTGCTGG              | 60   |
| 2   | OPA-20      | GTTGCGATCC              | 60   |
| 3   | OPB-01      | GTTTCGCTCC              | 60   |

2.8. Polymerase Chain Reaction (PCR)

PCR (Thermo Cycler) amplifications were performed in final reaction solutions 25 µl. A master mix for minimum of 3 samples and one control was prepared and aliquot of 21 µl placed in each PCR tube (Table 1). To make the final volume to 25 µl, four 4 µl of DNA template containing 50 ng/ µl was added to each tube.

Table 2. Polymerase chain reaction procedure

| No. | Material                  | Concentration | Amount  |
|-----|---------------------------|---------------|---------|
| 1   | Master mix(Amplicon)      | 2x            | 12.5 µl |
| 2   | RAPD Primer               | 20 Pico mole  | 2 µl    |
| 3   | DNase free water          | -             | 6.5 µl  |
| 4   | DNA template              | 50 ng/ µl     | 4 µl    |
|     | Total volume              |               | 25 µl   |

2.9. Genotypic Analysis

The RAPD bands were scored as present (1) or absent (0) in each pattern. All genetic parameters were estimated by GENEPOP software version,3.3 [25].

3. Result and Discussions

3.1. Effect of radish genotypes root aqueous extracts on germination and some seedling growth parameters of studied plant species.

The aqueous extracts of three tested of radish genotypes (red, black and white) were significantly affected on all studied traits (Fig.1). Highest values (73 %, 107.86 mg, and 63.43 mg respectively) were obtained by white radish for the germination percentage, total dry weight and plumule dry weight, whereas the lowest values (46.50 mg, 66.03 mg and 40.93) were measured by black radish for the same traits. Radicle dry weight reported highest value (38.33 mg) in white radish but the lowest value (24.33
mg) observed in red radish. The results of inhibition of germination and seedling growth inhibition in black radish revealed highest values (51.13% and 60.45%) and the lowest values (22.62 % and 35.85 %) accordingly for the same traits mentioned above in white radish.

Plumule length, plumule elongation velocity, seedling length, and seedling vigor index registered highest values (17.04 cm, 1.70 cm, 26.89 cm and 20.19 respectively) in white radish and the lowest values (10.94 cm, 1.09 cm, 15.98 cm and 11.00 respectively) documented of the mentioned traits in black radish. Also, radicle length and velocity of radicle elongation recorded highest values (9.85 cm, 0.98 cm respectively) recorded by white radish while for the same traits the lowest values (4.50 cm, and 0.37 cm respectively) in red radish (Fig.1). Results indicate that the allelopathic potential of black radish aqueous extracts was higher than red and white radish, germination of all studied plant species totally inhibited by aqueous extracts of black radish compare to white and red radish was more effective, Uremis et al. (2009) [8] found that black radish ensure higher suppression of Johnson grass than white radish and garden radish.

![Graph A](image1.png)

![Graph B](image2.png)

Figure 1. (A and B) Effects of radish aqueous extract on germination and some seedling parameters.

Where G% = germination Percentage I.O.G.= inhibition of germination, T.G.S=total germinated seed, TTS = total tested seeds, C.G.P. = control germination percentage, T.P.G = treatment germination percentage, PEV= plumule elongation velocity, TD= total days, RL= radicle Length, PL= plumule length , SL= seedling length (cm), SVI= seedlings vigor index.
3.2. Effect of Plant Species on Studied Characteristics

All crops and weeds were sensitive to the radish aqueous extracts, even though some were more sensitive than others; all parameters were significantly affected except seedling vigor index (Fig. 2). Highest values (60.88 % and 101.68 mg respectively) observed for germination percentage and total dry weight in bread wheat but the lowest values (55.56 % and 62.24 mg) for the same traits recorded in wild barley. Plumules dry weight reported the highest value (67.35 mg) in bread wheat, and the lowest value (38.02 mg) in wild oat. Inhibition of germination showed highest value (42.75 %) in durum wheat while the lowest values (34.37%) observed for the same traits in bread wheat. Radicle dry weight recorded the highest value (35.79 mg) in durum wheat and the lowest values (21.08 mg) in wild barley. The highest value (56.41 %) observed in wild oat and the lowest value (45.23 %) was with bread wheat. Whereas, radicle length and radicle elongation velocity documented the highest values (7.16 cm and 0.72 cm.day\(^{-1}\) respectively) in durum wheat but the lowest value (5.62 cm and 0.56 cm.day\(^{-1}\)) were with wild oat. Plumules length and plumules elongation velocity in wild barley registered the highest values (14.97 cm and 1.50 cm.day\(^{-1}\) respectively) but the lowest values (12.30 cm and 1.23 cm.day\(^{-1}\) respectively) were in bread wheat. Seedling length gained highest value (21.39 cm) in durum wheat but the lowest values (18.87 cm) was with wild oat.

All plant species were affected by root radish aqueous extracts, the most sensitive plant species was wild barley and bread wheat was more tolerant [10], who found that seed germination and seedling growth of wild barley were affected by allelopathic potential of black mustard. Germination and other seedling growth parameters in this study were negatively affected by water extract of radish root, in accordance to the results of [26] who indicated that hoary cress *Cardaria draba* water extract reduced germination of wheat, alfalfa, blue bunch wheatgrass, crested wheatgrass, and hoary cress when compared with distilled water, existence of phytotoxic chemical may inhibit germination percentage and other seedling growth.
Figure 2. (A and B) Effect of different concentration of radish aqueous extracts on studied plant characteristics.

Where G% = germination Percentage, G.S=Germination speed I.O.G.= inhibition of germination, T.G.S=total germinated seed, TTS = total tested seeds, C.G.P. = control germination percentage, T.P.G = treatment germination percentage, PEV= plumule elongation velocity, TD= total days, RL= radicle Length, PL= plumule length , SL= seedling length (cm), R./S=root/shoot SVI= seedlings vigor index.

3.3 Genetic Distance of Radish Species Using RAPD-PCR Technique.

DNA Quality and Quantifying

The concentrations and DNA purity of three samples of radish species was estimated by Nnodro spectrophometer. The concentration of samples was 300, 489 and 304 ng/µl for black, red and white radishes. The purity of DNA samples was between 1.71 to 1.86. Also, the quality of DNA was determined using 1% gel electrophoresis (Fig. 3). This verification show that the quality of DNA samples is very good and it is useful for future work and genetic analysis.

Figure 3. DNA extraction image by performing 1% agarose gel electrophoresis numbers stand as follow 1: black radish, 2: red radish and 3: white radish.

3.4. Genotypic Analysis

The RAPD bands were scored as present (1) or absent (0) in each pattern. All genetic parameters in present study were calculated by Genepop software, version, 3.3 [26]. There are various of molecular markers such as random amplified polymorphic DNA (RAPD) used to evaluate the genetic variances of radish speciesy. RAPD technique used because it is an effective in differentiation radish *Raphanus sativus* from other species of brassica. This is in agreement with [27] who explained that RAPD marker is effective to show dissimilarities among species of radish.

3.5. Number of Bands (NB)

The total of three primers amplified showed clear bands and to investigate the genetic variations among the three radish species (Black, Red, White) as shown in (Fig. 4).
Figure 4. Gel electrophoresis for three RAPD primers for three pooled radish samples P1: Black Radish; P2: Red Radish and P3: White Radish

The bands size range of the 3 primers over all the radish species, ranged from 180 to 2000 bp (Table 3). The smallest size of bands recorded for OPB-01 locus (180 bp in red and white radish species), while the highest size bands range was recorded for primer OPA-14 locus (2000 bp in black radish species). The results indicated that dissimilarities among the studied radish species, high fragments number found in black radish compared with the lowest fragments number in red radish.

| Primers Name | P1 (Black) | P2 (Red) | P3 (White) | Over All Bands |
|--------------|------------|----------|------------|----------------|
|               | No. of Band| Size range, bp | No. of Band| Size range, bp | No. of Band| Size range, bp | No. of Band| Size range, bp |
| OPA-14       | 4          | 400-1000  | 3          | 400-1000  | 3          | 750-1000  | 10         | 400-1000  |
| OPA-20       | 7          | 500-2000  | 6          | 500-1700  | 6          | 500-1700  | 19         | 500-2000  |
| OPB-01       | 8          | 180-1500  | 2          | 500-800   | 8          | 180-1500  | 18         | 180-1500  |
| Total        | 19         | 180-2000  | 11         | 400-17000 | 17         | 180-1700  | 47         | 180-2000  |

Table 3. Band numbers and bands size range (bp) radish species

3.6 Radish Species Gene Frequency

RAPD marker is a dominant marker it means the bands found or not, if yes the band symbol by 1, if not the band symbol by 0. The mean of gene frequency for all loci for allele 0 was 0.33 ranged from 0.00 for (OPA-20) loci to 0.33 for (OPA-14) loci to 0.67 for (OPB-01) locus and allele 1 reached 0.67, ranged from 0.33 for (OPA-01) locus to 0.67 for (OPA-14) loci to 1.00 for (OPA-20) locus (Table 4).

Table 4. Overall gene frequency of radish for all primers used

| No. | Locus | Allele 0 | Allele 1 |
|-----|-------|----------|----------|
|     |       |          |          |
3.7. Genetic Distance of Radish Species

When the same plant material was analyzed by RAPDs at the molecular level, a different pattern of relationships resulted among the accessions. Table (5) presents the Nei’s genetic distances among radish species. The genetic distance among the three studied radish species ranged from 17.33 to 37.6. The lowest genetic distance recorded between black and red that was 17.33 and the highest genetic distance recorded between black, red with white was 37.6 and white radish with all black and red radish were 65.067. These results are almost similar to the results of [28] when they used RAPD technique to estimate genetic distance between radish species and they found that high genetic distance was showed in local white radish and white radish Hybrid F1, but the minimum genetic distance was observed between local red radish and local white radish. Also, the results in this study were in agreement with the results of [15] which found a high genetic difference when they studying the diversity of thirteen radishes species in Pakistan.

Table 5. Overall Nei's genetic distance (below diagonal) Radish

| Radish types | Black | Red   | White |
|--------------|-------|-------|-------|
| Black        | ***   | 17.33 | ***   |
| Red          | 17.33 | ***   | 37.6  |
| White        | 37.6  | 37.6  | ***   |

3.8. Phylogenetic tree construction for radish species

As in the dendrogram below (Fig. 5), two clusters were generated, the 1st cluster was including both black radish and red radish species, and the 2nd cluster branch consisted of the white radish species. The most genetic similarity was between the black and red radish. It means the white radish genetically far from black and red radish.

Figure 5. UPGMA dendogram showing differentiation and relatedness among the radish species.
4. Conclusion

Aqueous extracts of black radish (*Raphanus sativus* L.) has high allelopathic impact on germination and growth development of bread wheat (*Triticum aestivum*), hard wheat (*Triticum durum*) crops and two endemic weed species wild oat (*Avena fatua*) and wild barely (*Hordeum spontaneum*), it could be used for inhibitions of these weed in early stage. Molecular experiment indicated that there is genetic diversity among three genotypes of radish species. The white radish genetically far from black and red radish. These results support the results of bioassay which indicates that the allelopathic effects of black and red radish species were more than the white radish.

References

[1] Molisch, H. (1937) Der Einfluss einer Pflanze auf die andere, Allelopathie. Fischer Jena.
[2] Rice, E. (1984) Allelopathy. 2nd (ed.) Acad. Press. Inc. Orlando. Florida, USA.
[3] Rice, E. L. (1995) Biological control of weeds and plant diseases: advances in applied allelopathy, University of Oklahoma Press.
[4] Weston, L. A. & Duke, S. O. (2003) Weed and crop allelopathy. *Critical Reviews in Plant Sciences.* 22(3-4) p.367-389.
[5] Rasul, S.A. and Ali, K.A., 2020 b. Study the Allelopathic Effect of Radish by Incorporate Into Soil on Some Poaceae Species. *Plant Archives.* 10(2), pp.3624-3627.
[6] Shaikh, F.K., Bradosty, S.W., Hamad, S.W. and Shinde, A.A., 2019. In Vitro Screening of Seed Extracts of Medicinal Plants for Protease Inhibitory Activity. *Cihan University-Erbil Scientific Journal.* 3(1), pp.61-65.
[7] Petersen, J., Belz, R., Walker, F. & Hurle, K. (2001) Weed suppression by release of isothiocyanates from turnip-rape mulch. *Agronomy Journal.* 93(1) p.37-43.
[8] Uremis, I., Arslan, M., Uludag, A. & Sangun, M. (2009) Allelopathic potentials of residues of 6 brassica species on johnsongrass [Sorghum halepense (L.) Pers.]. *African Journal of Biotechnology.* 8(15) p. 3497-3501.
[9] Rasul, S.A. and Ali, K.A., 2020. Effect of Radish Aqueous Extract on Germination and Seedling Growth of Wheat, Wild Oat and Wild barley. *Journal of Advanced Pharmacy Education & Research* Apr-Jun, 10(S2).
[10] Turk, M. & Tawala, A. (2003) Allelopathic effects of black mustard (Brassica nigra) on germination and growth of wild barley (Hordeum spontaneum). *Journal of Agronomy and Crop Science.* 189(5) p.298-303.
[11] Uludag, A., Uremis, I., Arslan, M. & Gozcu, D. 2005. Johnsongrass control using Brassicaceae crops. *4th MGPR Symposium.* P.21-24.
[12] Nomura, K., K. Yoneda, H. Uchiyama & T. Koyama, 1996. Growth characteristics of rat’s tail radish (*Raphanus sativus*) introduced from northern Thailand and chilling requirement for bolting and flowering. *Jap. J. Trop. Agric.* 40: 63–67 (In Japanese with English

---

[13] Kobayashi, K., Horisaki, A., Niikura, S. & Ohsawa, R. (2006) Inter-accession variation in floral morphology in radish (*Raphanus sativus* L.). *Euphytica.* 152(1) p.87-97.
[14] Matveeva, T. V., Simonova, A. V. & Lutova, L. A. 2002. Molecular markers of inbred radish (*Raphanus sativus* var. radicola Pers.) lines. *Cellular and Molecular Biology Letters.* 7(3) p.845-848.
[15] Rabbani, M. A., Murakami, Y., Kugimuki, Y. & Takayanagi, K. (1998) Genetic variation in radish (*Raphanus sativus* L.) germplasm from Pakistan using morphological traits and RAPDs. *Genetic Resources and Crop Evolution.* 45(4) p.307-316.
[16] Pradhan, A., YAN, G. & Plummer, J. 2004 Development of DNA fingerprinting keys for the identification of radish cultivars. *Australian Journal of Experimental Agriculture.* 44 (1), p.95-102.
[17] Williams, J. G., Kubelik, A. R., Livak, K. J., Rafalski, J. A. & Tingey, S. V. (1990) DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nucleic acids research.* 18(22), p.6531-6535.
[18] Welsh, J. & McClell and, M.1990. Fingerprinting genomes using PCR with arbitrary primers. *Nucleic acids research.* 18(24), p.7213-7218.
[19] Margale, E., Herve, Y., Hue, J. & Quiros, C. (1995) Determination of genetic variability by RAPD markers in cauliflower, cabbage and kale local cultivars from France. *Genetic Resources and Crop Evolution.* 42(3), p.281-289.
[20] Ali, K. A. (2016) Allelopathic potential of radish (Raphanus sativus L.) germination and growth of some crop and weed plants. *International Journal of Biosciences*. I.J.B.9 p.394-403.

[21] Naby, K.Y. and Ali, K.A., 2020. Effect of Sorghum [Sorghum Bicolor (L.) Moench] Aqueous Extract on Germination and Seedling Growth of Wheat, Wild Oat, Wild barley and Canary Grass. *Journal of Advanced Pharmacy Education & Research*. Apr-Jun, 10 (S2), p.191.

[22] Ali, K. & Aziz, F. (2002) Studying the effect of root and shoot extracts of syrian cephalaria (Cephalaria syriaca) extract on wheat seeds (Triticum aestivum) germination properties. *Zanco journal of pure and applied science*. 14 p.15-24.

[23] Siddiqua, S. A. (2013) Phenotypic effects of a fertility mutation in Norwgain White Sheep. Norwegian University of Life Sciences, Ås.

[24] Adefris, T. (2004) Diversity study based on quality traits, RAPD Markers, and Investigation of Heterosis in Ethiopian Mustard.* PhD. Thesis*. George-August University of Gottingen, Germany.

[25] Raymond, M. & Rousset, F. (1995) *An exact test for population differentiation*. *Evolution*. 49(6) p.1280-1283.

[26] KIEMNEC, G. L. & MCINNIS, M. (2002) Hoary cress (Cardaria draba) root extract reduces germination and root growth of five plant species. *Weed Technology*. 16 (1) p.231-234.

[27] CRUZ, S. M., NERY, M. C., VON PINHO, É. V. D. R. & LAIA, M. L. D. (2014) Molecular characterisation of radish cultivars. *Revista Ciência Agronômica*. 45(4) p.815-822.

[28] AL-Tufaili, A., Aboohanah, M., Salman, F. & Abdel-hassan, N. (2019) Analysis of Genetic Distance and Similarity in Some Radish Cultivars (Raphanus sativus L.) by Random Amplified Polymorphic DNA (RAPD) Markers. *Journal of Global Pharma Technology*. 11(3), P464-470.