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Integrated Management of Alternaria radicina in Carrot Seed Production: Efficacy of Fungicides and Plant Extracts

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ABSTRACT Carrot is an important crop used in fresh as well as processed form. Seeds can encounter various microorganisms during production, storage, and transportation. These microbes especially fungi, can cause infections that damages the seeds. The consequences of these fungal attacks include reduced germination rates, loss of vigour, shortened storage life, and physiological alterations that impact the seed's health and quality. Alternaria radicina is commonly found on carrot seeds. Whenever alone or associated with Alternaria dauci on the plant. Present research trials focussed on isolation of Alternaria pathogen and its integrated management. For this purpose, infected fruit samples were collected from Vegetable Research Institute and as well as from the local markets and associated pathogen were isolated and multiplied on artificial growth medium. Management trials were performed by using different fungicides through poisoned food technique. Different plant extracts namely Datura, Garlic, Neem and Safeda and chemicals namely Hombre, Cobox, Menzob and Carbendazim will be evaluated. Results of all the experiments of fungicides and plant extracts revealed that out of four tested fungicides and plant extracts Cobox and Garlic gives best results against A. radicina mycelial growth and disease control. Results also showed that deep red variety is highly resistance of A. radicina and garlic extract was effective at all studied concentration. This variety may be beneficial in the future against the A. radicina pathogen in carrot breeding.

Keywords: *Carrot; Germination; Fungicides; Garlic; Microorganisms*

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INTRODUCTION Carrot (*Daucus carota* L.) is an important crop mostly used in fresh form and sometimes processed. It belongs to the family Apiaceae. “Daucus” consist of 25 species and is presently considered as leading genus in the umbelliferea family (Hasan et al., 2005). Carrots is a biennial crop with a diploid chromosomal number that are grown in many different climates and are an essential food source worldwide, particularly in the fight against vitamin A deficiency in children. Originally used as a medicinal herb, their relevance comes from their vast variety of bioactive components, such as falcarinol, which is thought to lower the risk of cancer, and their quantity of carotenoids, which act as a natural precursor to vitamin A (Kobæk-Larsen et al., 2005). China is top ranked country for carrot production with 19 million tonnes out of 37 million tonnes total world production (FAO., 2013). Advanced countries other than China that produce carrots are Denmark (44.29 tonnes per hectare), Belgium (47.64 tonnes per hectare), and the United Kingdom (44.28 tonnes per hectare). The total cultivated area of carrot in Pakistan is 29038 ha, with a production of 509066 tonnes; it generates a large amount of revenue in terms of vegetable export to many foreign countries; 150,228 metric tonnes of carrot were exported in the year (2006), contributing about 180,163 US dollars to Pakistan's economy, and this share increased by 39% from 2007 to 2011. Vegetables demand a moderate temperature range as well as fertile soils for good productivity. Fortunately, Pakistani agricultural land is ideal for for higher yields every year and export of vegetable. The vegetable usually contains 12.9% protein, 59.40% carbohydrates and 2.9 % fibres (Kumar and Ali, 2000).

Carrots are produced by seed, there are over ten diseases that are spread by seed. Alternaria radicina caused Alternaria blight. This is a serious infection that affects carrot seed and is a restrictive

reason for the crop (Farrar et al., 2004). According to scientist carrot Alternaria Blight was originally recorded in Germany in 1855 and afterwards from various temperate and Mediterranean countries such as India, France, Belgium, Holland, the United States, Denmark and Israel (Jensen, Knudsen, Madsen, & Jensen, 2004). Alternaria blight is thought to be spread by contaminated and infected seed. The rate of inoculation, host susceptibility, leaf wetness duration, and field temperature are all factors that influence pathogen transmission.

Seed during production, storage and transport are exposed to various form of microorganism that leads to fungal infections and harmfully affect it by reducing germination and vigour, shortening the storage period, and promoting physiological changes (Baka et al., 2014). A.radicina is a seed borne fungal pathogen that causes diseases in carrots and has a negative effect on the crop. Around the world, the pathogen is responsible of losses in field, post harvested condition and in yield. When a pathogen affects a crop, it produces a variety of symptoms, including lesions that are black and sunken in appearance and the disease spreads rapidly up to 62 percent under moist conditions. High levels of infestation have been found by A. radicina and carrot seed contamination due to this is a permanent issue. A. radicina or A. dauci mild or heavily infested seeds were not able to germinate or the hypocotyls of these seeds at above or beneath the soil line are infested, causing post development damping off inside 2 to 3 weeks after the emergence (Pryor and Strandberg 2002). Seed-borne fungal infections, which can drastically affect seed quality and spread quickly through the air, rain, and diseased plant stubbles, primarily obtain their inoculum from contaminated seeds.

Using certified seeds and applying seed treatments like hot water treatment or fungicides like A. radicina for targeted eradication within a certain timeframe are effective control strategies that are essential for managing these persistent infections. Plant-derived products have a lot of success as an alternate strategy for plant disease management in recent years (Arya and Perelló, 2010). Essential oils and plant extracts appeared to be effective at controlling pathogens found in seeds (Schwan-Estrad et al., 2003). Plant extracts have been shown to be effective in inhibiting seed-borne diseases as well as improving seed quality and seed embryo emergence (Nwachukwu and Umechuruba, 2001).

The purpose of this study is to investigate the management of Alternaria radicina, a prevalent pathogen that affects the development of carrot seeds, and the efficacy of fungicides and plant extracts. By conducting extensive study, we hope to evaluate the integrated management approaches that can be used to lessen Alternaria radicina effects and improve the quality and yield of carrot seeds.

MATERIAL AND METHODS

Carrot seeds of Red Lady, Deep Red, Red Core, Anmol, Neelam, T-29, Proline and Desi varieties were collected from Vegetable Research Institute, AARI Faisalabad, IHS, UAF. For evaluation of seed health status, blotter paper method and potato dextrose agar methods were used.

Inoculation of Carrot seeds with Alternaria radicina

To inoculate seeds of carrots were artificially expose to the pure culture of A. radicina in petri dishes. After this procedure, seeds were treated with various chemicals as well as plant extracts and were raised in plastic pots or trays, to determine the expression of symptomology and on daily basis germination of seedlings were observed.

Identification of fungi

When growth was observed on the 9th day, isolated fungi were identified and purified using literature, based on growth patterns, colony colour and sporulation type of mycelium of associated mycoflora may be easily detected by a review of the literature as well as through Dematiaceous hyphomycetes. A review of previously published work was used to investigate the culture of isolated mycoflora. He identified the single spore approach to examine post-detected infections.

Detection of Associated Mycoflora by Seed Testing Methods:

The maximum seed mycoflora from carrot seed was determined using two methods: the Agar Plate Method and the Standard Blotter Paper Method.

Integrated Management for Associated Mycoflora

In-vitro Management through fungicides

By using food poisoned technique, the efficacy of the fungicides was tested. In this method, in petri dishes fungicides at 200, 400 and 600 ppm concentration were dissolved in PDA media. Petri dishes comprising of agar media were consider control. To identify the inhibitory effect of mycelium, hyphal tip of pure culture of pathogen was shifted in the center of the 9mm petri dish. On the basis of fungal mycelia growth on media was recorded after 4-5 days to doing the inhibitory action of fungicides (Haque et al., 2007). To assess the effect of fungicides, four different fungicides namely Hombre, Cobox, Mencozab and Carbendazim were applied against all varieties as a seed dresser, to determine which fungicide shows good germination percentage; to control the seed borne mycoflora. The Sand Tray Methods were used to conduct an in vitro management evaluation of these four fungicides to determine the germination percentage.

The percentages (percent) of seed germination were calculated

In-Vitro evaluation of plant extracts

Garlic (Allium sativum), Safeda (Eucalyptus comaldulances), Neem (Azadirachata indica) and Datura (Datura strumarium) extracts were evaluated against Alternaria radicina. In the laboratory the efficacy of four plant extracts was examined at three concentration levels viz., S, S25 and S50% via poisoned food technique on PDA. All treatment was replicated five times. To identify the inhibitory effect of mycelium, hyphal tip of pure culture of pathogen will be shifted in the centre of the 9mm petri dish. The mycelial development of the fungus was verified after 2 days, 5 days and 7 days of incubation.

Statistical analysis

Under in-vitro condition recorded facts and data of microbial growth under CRD test will be determined. Through the use of computer software STATISTIX 8.1, significant treatments and their combination for the control of seed borne pathogens of carrot will be identified (Strandberg, 1987).

RESULTS AND DISCUSSION

During the years 2019-2021, the current research was carried out at the Department of Plant Pathology's Seed Health Testing Lab. The purpose of this study was to discover and manage fungus in carrot seeds and seedlings. Data on numerous parameters was gathered and statistically examined using analysis of variance procedures at a significance level of 0.05 percent.

The pathogenic fungi that cause carrot Alternaria blight were A. radicina, A. dauci and A. alternata. All eight varieties were affected to various levels and indicated variable percentages of infestation. Table 1 clearly showed that Red Lady was infested with A. radicina (23.41%), A. dauci (18.92%) and A. alternata (17.47%), Neelam showed (24.56%), (17.38%) and (18.69), Red core showed (21.51%), (18.36%) and (17.67%), Deep red showed (13.1%), (16.9%) and (18%), Anmol showed (22.7%), (18%) and (17.1), T-29 showed (22.23%), (18.4%) and (15.3%), Proline showed (24.9%), (18.6%) and (16.8%), and Desi variety showed (25.5%), (19.41%) and (19.4%). According to the percentage ratio of mycoflora, Deep red showed a slight resistance response as compared to others and Desi variety had the most pathogenic fungal association.

Biochemical Management of Alternaria radicina

After 2, 5, and 7 days of incubation, the fungus' mycelial growth was measured. The percentage of inhibition of mycelial growth was calculated and the results revealed that Garlic extract was the most effective in inhibiting mycelial growth of Alternaria radicina (49.54, 58.67, and 73.90 percent) at S/50, S/25 and S % after seven days followed by Neem leaf extract (32.77, 44.97, and 61.73 percent) over control. At S/50 percent, Safeda Leaf Extract gave least inhibition percentage of mycelial growth Alternaria radicina over control (13.61%) at S/50 % as mentioned in Table 4

Effect of Plant Extracts recorded after 2 days

After two days of incubation, the mycelial growth was measured and compared to the control. The percentage of mycelial growth inhibition was computed for each treatment and the mean values were given in Table 4. After two days, the plates' inhibition was calculated and statistically investigated. The p-value revealed that the findings were highly significant, indicating that at least one of the treatments used did not have the same effect. The Tukey HSD All-Pairwise Comparisons Test was used to see whether treatment performed better as mentioned in Table 6. The findings were interpreted using statistical analysis.

Effect of Plant Extracts recorded after 5 day

The mycelial growth on the petri plates was measured and compared to the control after five days of incubation. The percentage of mycelial growth inhibition was computed for each treatment and the results were presented in the Table 4.

According to the data, Garlic had the highest percentage growth inhibition (53.17 percent) at S concentration, followed by Neem (43.47 percent) at the same concentration. In the case of Safeda, the minimum percent growth inhibition was 9.59 percent at S/50 concentration.

The plates were examined again after five days and inhibition was computed and statistically assessed. Table 6 described the Tukeys HSD All-Pairwise Comparisons Test was used to determine which treatment performed better. The findings were interpreted using statistical analysis.

Effect of Plant Extracts recorded after 7 days

After seven days, the petri plates were examined again and the inhibition % of mycelium growth was computed and the following data was recorded.

The foregoing data showed that among the four included extract, Garlic had the highest percentage growth inhibition (73.906%) at S concentration, followed by Neem (61.732%) at S concentration. In the case of Safeda, the minimum percent growth inhibition was 13.618 percent at S/50 percent concentration.

The plates were examined again after seven days and inhibition was computed and statistically assessed. The p-value revealed that the findings were highly significant, indicating that at least one of the treatments used did not have the same effect. . The Tukey HSD All-Pairwise Comparisons Test was used to determine which treatment performed better. The findings were interpreted using statistical analysis as in Table 5.

Carrot production is a popular issue in any carrot-producing country but it has been severely harmed by unhealthy seeds because a good seed will always produce a good crop. Bacteria, nematodes, biotic and abiotic factors all harmed the goal of high yielding crops. Aflatoxins have been a major issue in recent years as a result of their destructive approaches to crop production and human health. A. radicina is a seed borne fungal pathogen that causes diseases in carrots and has a negative impact on the crop. Pathogens caused diseases in the field, post-harvest conditions and production losses have all been observed around the world. When a pathogen infects crops, it causes various symptoms such as lesions, which are black sunken forms and infection spreads rapidly up to 62 percent in moist conditions. The fungal pathogen Alternaria dauci has also been identified as the cause of carrot leaf blight. It is one of the most common foliar diseases of carrots in the world (Gugino et al., 2004). A. dauci develops an irregular lesion on leaflets that is brown to black in colour (Farrar et al., 2004).

In Pakistan, Alternaria root rot and fungal leaf blight are recognized to exist. On carrot seeds, found Stemphylium botryosum, Botrytis cineria, Fusarium solani, Altemaria radicina, Aspergillus flavus and Alternaria altemata.

The scientist investigated that, the seed health of vegetables such as turnip, carrot, bottle gourd, cabbage, spinach, sweet gourd, red amaranth and reddish, which were collected from various locations and tested for seed-borne mycoflora such as Penicillium spp., Rhizopus spp., Alternaria spp., Chaetomium funicola, Curvularia spp, A. niger, A. flavus and Phoma spp.

Seed treatment is the only way to prevent diseases spread by seeds. When crops are harvested, fungicides suppress the black rot disease and they also provide excellent protection during post- or pre-storage conditions. When the four fungicides Cobox (copper oxychloride), Mancozeb, Hombre, and Carbendazim were used as seed dressers to determine the percentage germination, the results revealed that Cobox (copper oxychloride) had the highest mean percentage of germination

(80.083%), Hombre (Tebuconazole) had the second highest mean percentage of germination (52.250%), and Carbendazim had the third highest (Davis and Raid, 2002). Previously scientists investigated the effect of leaf extract on spore germination of some of the most common Daucus carota fungal infections in vitro. Antifungal activity of leaf extracts of Strychnos nux-vomica I, Allium cepa, Azadirachta indica, Occimum sanctum, and Allium sativum at 50, 75, and 100 ppm concentrations against Alternaria dauci, A.radicina, Botrytis cinerea, Cercospora carotae, and Sclerotium rolfsii in root vegetables.

Table 1: Mean Incidence (%)

Fungi	Red Lady	Neelam	Red core	Deep Red	Anmol	T.29	Proline	Desi
A. radicina	23.41	24.56	21.51	13.1	22.7	22.33	24.9	25.5
A. dauci	18.92	17.38	18.36	16.9	18	18.4	18.6	19.41
A. alternata	17.47	18.69	17.67	18	17.1	15.3	16.8	19.4

Table 2: Analysis of variance (ANOVA) for all varieties

S.V	DF	Red lady	Neelam	Red core	Anmol	Deep Red	T-29	Desi	Proline
Replications	2	0.531	0.304	0.412	0.673	4.3531	1.934	0.437	5.082
Methods	1	128.17	296.006	295.369	264.554	62.196	175.155	181.21	213.968
Fungus	6	209.726	189.919	70.474	159.16	83.8401	149.456	228.7	174.753
Methods X Fungi	6	28.617	76.879	17.833	43.98	15.6195	14.816	11.806	46.445
Error	26	1.956	0.721	1.669	1.067	1.2581	0.639	1.539	0.907
Total	41								

Table 3: Comparison of Mean values for all varieties

Fungus	Red lady	Neelam	Red core	Anmol	Deep Red	T-29	Desi	Proline
Alternaria radicina	23.413 A	24.562 A	21.517 A	22.895 A	17.773 AB	22.233 A	25.487 A	24.917 A
Alternaria dauci	18.923 B	17.382 C	18.360 B	18.037 B	16.900 B	18.445 B	19.413 B	18.610 B
Alternaria alternate	17.478 B	18.692 B	17.677 B	17.850 B	18.310 A	15.933 C	19.477 B	16.875 C
Rhizopus spp.	13.165 C	14.543 E	15.072 C	13.732 C	16.822 B	13.682 D	17.023 C	12.792 E
Fusarium spp.	17.672 B	13.362 F	14.688 C	13.293 C	12.908 C	12.142 E	14.302 D	13.943 D
Aspergillus spp.	9.465 D	15.788 D	14.245 C	14.377 C	11.645 C	9.313 F	10.512 E	14.860 D
Verticillium spp.	6.252 E	6.065 G	10.898 D	6.345 D	8.417 D	8.237 G	6.993 F	7.470 F
Total								

Table 4: Percentage growth inhibition by Plant extracts after 2, 5 and 7 Days

Treatment	Concentrations	Growth Inhibition % (after 2 days)	Growth inhibition % (after 5 days)	Growth Inhibition % (after 7 days)
Datura	S/25	9.798	18.798	26.128
Datura	S	7.894	23.328	32.428
Datura	S/50	5.558	13.236	18.398
Garlic	S/25	18.128	42.216	58.676
Garlic	S	22.832	53.17	73.906
Garlic	S/50	15.308	35.642	49.542
Neem	S/25	13.302	31.67	44.972
Neem	S	18.258	43.474	61.732
Neem	S/50	9.694	23.08	32.774
Safeda	S/25	6.072	15.036	21.348
Safeda	S	7.864	19.468	27.648
Safeda	S/50	3.872	9.59	13.618

Table 5: Completely Randomized ANNOVA for Inhibition after 2, 5 and 7 days

Source	DF	(MS) 2 days	(MS) 5 days	(MS) 7 days
Treatment	11	177.761	941.355	1828.19
Error	48	0.2	1.139	2.25
Total	59			
Grand Mean		11.548	27.392	38.431
CV		3.87	3.9	3.9

Table 6: Tukeys HSD All-Pairwise Comparisons Test of Inhibition by Treatment after 2, 5 and 7 days

Treatment	Concentrations	Homogeneous Groups	After 2 days	After 5 days	After 7 days
Datura	S/25	F	9.798	18.798	26.128
Datura	S	E	7.894	23.328	32.428
Datura	S/50	G	5.558	13.236	18.398
Garlic	S/25	B	18.128	42.216	58.676
Garlic	S	A	22.832	53.17	73.906
Garlic	S/50	C	15.308	35.642	49.542
Neem	S/25	D	13.302	31.67	44.972
Neem	S	B	18.258	43.474	61.732
Neem	S/50	E	9.694	23.08	32.774
Safeda	S/25	G	6.072	15.036	21.348
Safeda	S	F	7.864	19.468	27.648
Safeda	S/50	H	3.872	9.59	13.618
Alpha			0.05	0.05	0.05
Tukey HSD Value			0.9702	2.317	3.2551

CONCLUSION

The fungicides and plant extract showed the potential to control A. alternata and A. dauci, since their lower concentrations were able to satisfactorily reduce the incidence of these fungi. They were harmless to carrot seed germination and emergence. Through a systematic investigation into the efficacy of fungicides and plant extracts, valuable insights have been gained regarding their effectiveness in mitigating the impact of this destructive pathogen. The findings highlight the potential of integrating both chemical and natural approaches to achieve sustainable disease management while minimizing environmental impact. However, further research is warranted to optimize the application techniques and explore additional control measures for enhanced efficacy. By adopting integrated management practices, carrot seed producers can mitigate the economic losses caused by Alternaria radicina and contribute to the long-term sustainability of carrot cultivation.

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