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ECO-FRIENDLY APPROACHES FOR THE MANAGEMENT OF WHEAT SEED-BORNE FUNGI

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Abstract
A laboratory experiment was carried out to evaluate the efficacy of the same plant extracts against seed-borne fungi isolated from wheat. Leaf extracts of three plants(Thyme, Castor, Mint) , the highest in the habitation of 75% castor (52.5)mm colony diameter comparison with anther treatments Leaf extract of castor 0.75% caused the highest inhibition of mycelial growth of *Rhizoctonia solani* (46.38) %, while the lowest inhibition 2.08%) of mycelial growth was measured at 0.25 leaf extract of thyme, 0.50% of castor and (0.50 , 0. of mint as compared to control. effect of plant extracts on seedlings in the greenhouse They had a significant effect between treatments comparison with the positive control the , highest number of post-emergence damping off recorded of fungi *Arthriniumarundins*was1.25 and lowest number of post-emergence damping off represented of all treatments by plant extracts (thyme, castor, mint) was (0)and fungicides (Pristine^{tm} WG).highest degree of diseases severity was 7.75 comparisons with another treatment of castor plant extract they had lowest inhabitation of fungi *curvularia sp* the lowest degree of diseases severity of fungi *Arthrinium arundins , Aurobasidium pullulans* and *Alternaria aalternata* was 1.25 in mint plant extract and fungicides respectively. The highest percentage of seed germination in the pots treated with the thyme plant extract had the fungicide 100 % and recorded the lowest percentage of seed germination the mint plant extract treated at 55%. The maximum shoot length and root were obtained in castor 24.87cm shoot length in treatment inoculated by *Rhizoctonia solani* root length treatment 14.50cm in the negative control treatment minimum shoot length castor treatment 14.87 cm in castor inoculated by *Curvularia*sp and root length 7.37 cm in mint treated was inoculated by *Aurobasidiumpullulans*and in positive control, treatment was inoculated by *Aurobasidiumpullulans*and*Nigrosporasphaerica.* the high fresh weight of Pristine^{tm}WG) 1.40 gm inoculated by *Curvularia*spand root weight 0.33 gm inPristine^{tm} WG) treated inoculated by *Fusarium solani* and low weight of length 0.79gm of mint treated, inoculated by *Alternaria alternata* and root weight 0.02 gm in thyme treated inoculated by *Fusarium solani*.

Keywords: Antifungal activity, plant extracts; seed treatment, biocontrol agents seed borne fungi.

1. Introduction

Numerous plant-derived compounds have been shown to have antifungal action against a variety of phytopathogens. Additionally, it was discovered that various medicinal and herbaceous plants' antifungal characteristics were effective against mycoflora carried by grain seeds(Yassin et al., 2012, Kiran et al., 2010). Garlic is especially promising among the several plant compounds often employed to combat seed-borne mycoflora due to its very substantial antifungal activity and antibacterial characteristics (Aqil et al., 2010, Reddy et al., 2010).

To stop seed-borne fungus, seedling blight, damping-off, and seed deterioration, seed dressing fungicides have additionally been utilized for cereal seeds (Kadege et al., 2015). Although using synthetic chemical fungicides may effectively and efficiently control seed-borne fungus, this could not be done with grains owing to pesticide toxicity (Kadege et al., 2015). It is now understood that chemical fungicides are hazardous to non-target species and generate major environmental issues(Kadege et al., 2015). The most effective and affordable method to manage seed-borne fungal diseases is seed treatment(CHANDLER, 2005).Biological and fungicides control agents are used in connection to this as seed treatments or seed priming. The microbes known as "biocontrol agents" guard seedlings and seeds against a variety of seed-borne fungus(Reddy, 2012, Pawar et al., 2015).

Fungicides have been used to control these diseases with very little effectiveness, and their continued use is uncertain as a result of tighter regulatory requirements. Therefore, the current strategy for controlling plant diseases focuses on reducing the use of fungicides to reduce environmental pollution and discovering alternatives to chemical fungicides. Since plant metabolites are eco-friendly and botanicals play a significant role in plant metabolites, their application for the treatment of plant diseases has recently become a vital part of Integrated Pest Management (Sahayaraj et al., 2009).Numerous research investigations have been carried out to examine the biological components and antifungal characteristics of various plants (Stephan et al., 2005, Satish et al., 2010). Some essential oils or plant water extracts were shown to have a very substantial antifungal action, according to studies. Given that certain plants are already recognized to have a variety of biological functions(Amin et al., 2009, Belabid et al., 2010).

The objective of the present study the effect of leaf extracts of mint *Mentha piperita*(Lamiaceaefamily) , Castor (Ricinus communis L.)) and Thymus on major isolated wheat seed borne fungi; and on seedling emergence, seed germination, diseases severity , length of plant , fresh and dry weight of plant were investigated.

2. Material method

2.1 Preparation plant extracts

10 grams of every sun-dried medicinal plant material were divided into smaller pieces and macerated using a blender until they were 1-2 mm in size. The resulting powder was then combined with 100 ml of distilled water. was put at room temperature 24 hours for . The resulting extract

filtered by Buckner funnel then rinsed with a small amount (about 30 ml) of 96% ethyl alcohol. were the extract solutions were evaporated at 40 °C in the oven. dried extraction kept in the freezing refrigerator (Bokhari, 2009) then prepared different concentration (0,0.25, 0.50,0.75)gm/100ml from dried extraction (Abdlrahmaan et al., 2018).

2.2 Botanicals Antifungal activity on the fungi growth by poison food method

Leaf extracts of three plant samples (thyme , castor , mint), were evaluated against mycelial growth of isolated of wheat seeds *Aurobasidiu mpullulans , Curvularia sp , Fusarium solani , Alternaria alternata , Arthrinium arundinis,Rhizoctonia solani , Nigrospora spaerica*.

Prepared four concentration of each plant extract include (0.0.25,0.50, 0.75)gm/100ml PDA media. After sterilization by autoclave poured 20 ml in to sterilized Petri plates allowed to solidify, Utilizing the sterile cork borer, 0.4 mm disk of test fungus 8–10 day's old culture plate and was put in the middle of a petri-plates. Every treatment has three replicated. The inoculated plates underwent a 10-day incubation period at 28 °C. According to Vincent's formula, the percentage inhibition of mycelial growth was computed.

$$I = \frac{C - T}{C} \times 100$$

Where I= Percent Inhibition; C= Growth of pathogen in control and T= Growth of pathogen in treatment.(Ahmad et al., 2016)were autoclaved for 20 min at121/bar(Derbalah et al., 2012)

2.3 Effect of plant extracts on seedlings in the greenhouse

Wheat cultivars' untreated and treated with plant extract grains were employed in the tests. Separate water extracts were used to soak the wheat grains for one hours in the three (thyme , castor , mint) plant extracts 0.75 gm/ 100 water and one fungicide (Pristine^{tm} WG (active ingredients : Boscalid 25.2% and Pyraclostrobin 12.8%) ,(Formula :water dispersible granules) , (Origin: BASF USA)) 0.25gm/100ml water , were also included in the assay for comparison purposes, and then treated wheat seeds plated in a sterilized soil mix with isolated fungi in pots. The untreated grains served as the control by plating in only sterile soil and wheat seed being soaked in distilled water for an hour. For each extract and fungicide 140 grains in total were steeped (Baka, 2014).

In the soil approach, 5 grains were evenly spaced out in each pot and planted at a depth of 2.0 cm. There were four replications of each test.

The seedling emergence was recorded in grains sown in the sterilized soil mix with seven pathogens (*Aurobasidium pullulans , Curvularia sp , Fusarium solani , Alternaria alternata , Arthrinium arundinis ,Rhizoctonia solani , Nigrospora spaerica*.) for all treatments instead the negative control without pathogen . After two weeks , measured the germination of wheat seed when the first leaf of the seedling reached .The shoot, lengths root, severity of diseases .the result showed of diseases severity modified 0-9 scale for yellow spot reaction in wheat plant the diseases expression recorded (Dinglasan et al., 2016), post emergence damping off . In addition, fresh and

dry weight of germinated seedlings were measured (Baka, 2014).Relative frequencies of percent germination of seeds was calculated.(Ahmad et al., 2016).

$$\%\ germination\ = \frac{No.of\ seeds\ gerninated}{Total\ number\ of\ seeds} \times 100$$

2.4 Statistical analysis

After checking the data for normality, analysis of variance (ANOVA) was carried out. Duncan's multiple range test was used to compare means (Duncan, 1975)at $p \leq 0.01$ by the statistical analysis software IBM SPSS statistics (v28) according to one-way ANOVA (Basto and Pereira, 2012).

3. Result and discussion

3.1 Antifungal assay

In the current research, the potential of aqueous extracts prepared from selected plants to inhibit the mycelial growth of three fungi by Poisoned food technique. Poisoned food technique has been extensively used by several researchers to investigate antifungal potential of plant extracts against a range of phytopathogenic fungi ((Khan and Nasreen, 2010),(Farooq et al., 2010) ,(Ngadze, 2014), (Omidpanah et al., 2015) ,(Kekuda et al., 2016)).

The result of inhibitory potential of plant extracts of selected plants(thyme, castor, mint)was shown in (Fig.1) the inhibition of mycelial growth of tested fungi , highest in habitation of 75% castor (52.5)mm colony diameter comparison with anther treatments, castor have significant effects on the isolated wheat seed borne fungi.

The findings illustrated in Table 1 confirmed that leaf extracts of all three plant extracts (thyme, castor, mint)significantly inhibited mycelial growth of (*Aurobasidium pullulans , Curvularia sp , Fusarium solani , Alternaria alternata , Arthrinium arundinis ,Rhizoctonia solani, Nigrospora spaerica*.) Leaf extract of castor 0.75% caused highest inhibition of mycelial growth of *Rhizoctonia solani is* (46.38) %followed by *Rhizoctonia solani*(43.76%) and *Nigrospora spaerica* 30.21%, while the lowest inhibition 2.08%) of mycelial growth was measured at 0.25 leaf extract of thyme , 0.50% 0f castor and (0.50 , 0. of mint as compared to control.

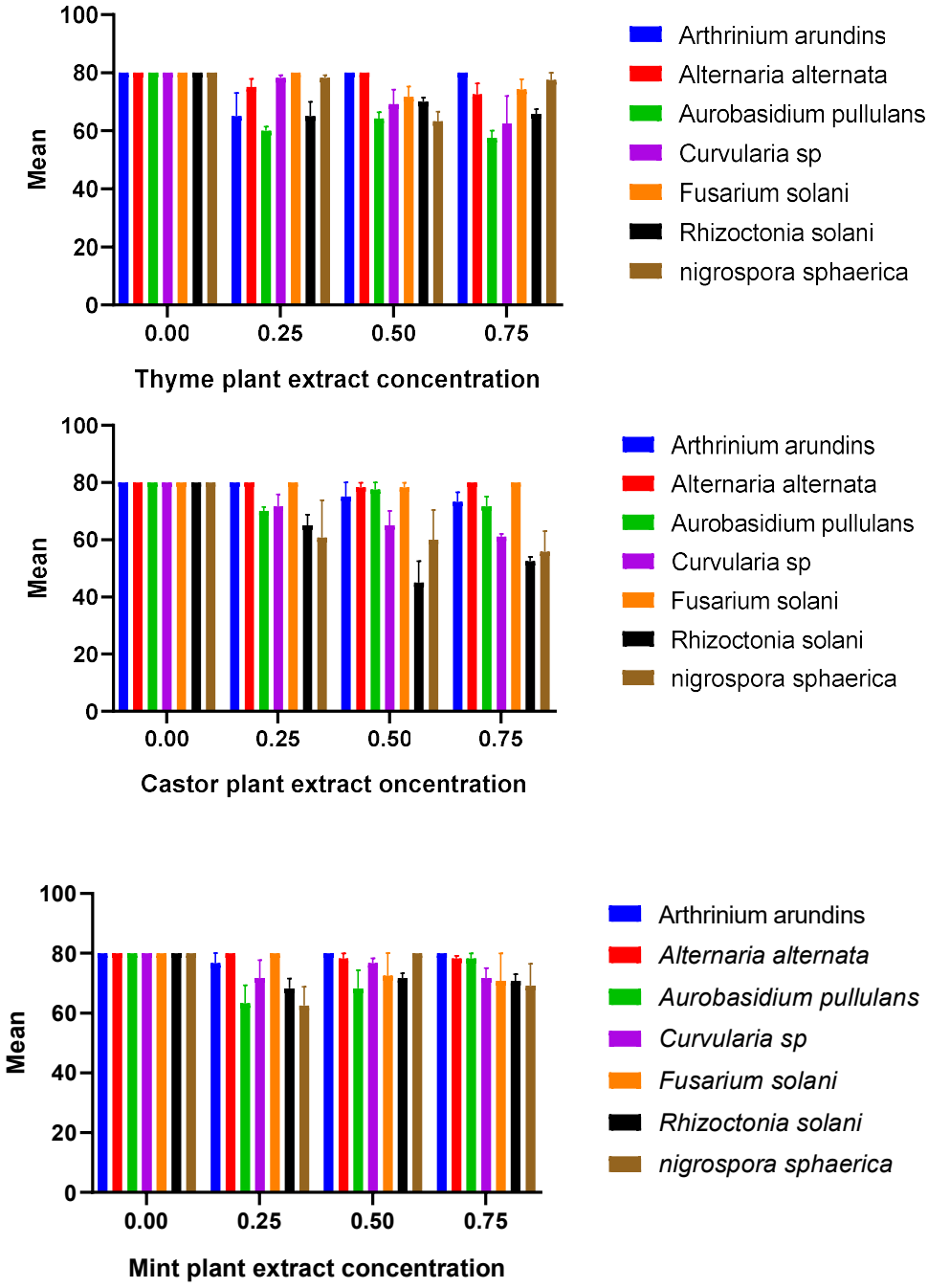

Fig . 1. Mean of colony dimeter (mm) and mean standard errors, In vitro evaluation of plant extracts (Thyme , Castor , Mint) against isolated seed born f

Table 1. Percentage of Water plant extracts at varied concentrations inhibiting the mycelial development of tested fungus

Isolated Fungi of seed wheat		*Arthriniu marundins*	*Alternaria alternata*	*Aurobasidium pullulans*	*Curvulariasp*	*Fusarium solani*	*Rhizoctonia solani*	*Nigrosporasph aerica*
Thyme	0%	0^{g}	0^{g}	0^{g}	0^{g}	0^{g}	0^{g}	0^{g}
	25%	14.54^{c-g}	6.25^{efg}	25.00^{b-f}	2.09^{g}	0^{g}	18.75^{c-g}	2.09^{g}
	50%	0^{g}	0^{g}	19.79^{c-g}	13.54^{c-g}	10.42c-g	12.50^{c-g}	20.83^{c-g}
	75%	0^{g}	8.33^{c-g}	29.17^{a-d}	21.87^{c-g}	7.29^{d-g}	14.59^{c-g}	3.13^{f-g}
Castor	0%	0^{g}	0^{g}	0^{g}	0^{g}	0^{g}	0^{g}	0^{g}
	25%	0^{g}	0^{g}	12.50^{c-g}	11.46^{c-g}	0^{g}	18.75^{c-g}	25.00^{b-f}
	50%	6.25^{efg}	2.08^{g}	2.08^{g}	12.5^{c-g}	2.08g	43.76^{ab}	26.04^{b-e}
	75%	8.33^{c-g}	0^{g}	10.42^{c-g}	19.58^{c-g}	0^{g}	46.38^{a}	30.21^{abc}
Mint	0%	0^{g}	0^{g}	0^{g}	0^{g}	0^{g}	0^{g}	0^{g}
	25%	4.17^{efg}	0^{g}	20.83^{c-g}	10.42^{c-g}	0^{g}	14.58^{c-g}	21.87^{c-g}
	50%	0^{g}	2.08^{g}	14.58^{c-g}	4.17^{efg}	9.37^{c-g}	8.33^{c-g}	0^{g}
	75%	0^{g}	2.09^{g}	2.08^{g}	10.42^{c-g}	11.46^{c-g}	14.59^{c-g}	13.54^{c-g}

Means had a common letter in the columns are non-significant different at $p \leq 0.01$ as analyzed by Duncan

3.2 Effect of plant extracts on seedlings in the greenhouse

The inhibitory impact of the tested plant extracts of means of post emergence damping off (table 2) they had significant effect between treatments comparison with positive control highest number of post emergence damping off recorded of fungi *Arthriniumarundins*1.25 and lowest number of post emergence damping off represented of all treatments by plant extracts(thyme,castor,mint)and fungicides (Pristine^{tm} WG).

Table.2The effect of plant extract (Thyme , castore , Mint) and fungicide (Pristine ^{tm} WG)on wheat cultivar shows symptom of post emergence damping off of seedlings

Fungal isolates	Negative control	Positive control	(Pristine^{tm} WG) 0.25%	Thyme 0.75%	Castor 0.75%	Mint 0.75%
Arthriniumarundins	0^{b}	1.25^{a}	0.50^{ab}	0.25^{ab}	0.50^{ab}	0.25^{ab}
Alternaria alternata	0^{b}	1.00^{ab}	0.25^{ab}	0^{b}	0.50^{ab}	0^{b}
Aurobasidium pullulans	0^{b}	0.25^{ab}	0.25^{ab}	0.50^{ab}	0.25^{ab}	0.50^{ab}
*Curvularia*sp	0^{b}	0.25^{ab}	0.5^{ab}	0^{b}	0.50^{ab}	0.25^{ab}
Fusarium solani	0^{b}	1.25^{a}	0.25^{ab}	0^{b}	0^{b}	0^{b}
Rhizoctonia solani	0^{b}	0.50^{ab}	0.25^{ab}	0.25^{ab}	0^{b}	0^{b}
Nigrosporasphaerica	0^{b}	0.50^{ab}	0^{b}	0.50^{ab}	0.50^{ab}	0^{b}

Means had a common letter in the columns are non-significant different at $p \leq 0.01$ as analyzed by Duncan

In the Fig.2 shows castor plant extract they had lowest inhabitation of fungi *curvularia sp* because recorded the highest degree of diseases severity 7.75 comparison with anther treatments , the mint plant extract was have the same effect with fungicide (Pristine^{tm} WG) for decrease the effect of seed borne pathogens , the lowest degree of diseases severity of fungi *Arthrinium arundins* , *Aurobasidium pullulans* and *Alternaria alternata* 1.25 in mint plant extract and fungicides respectively.

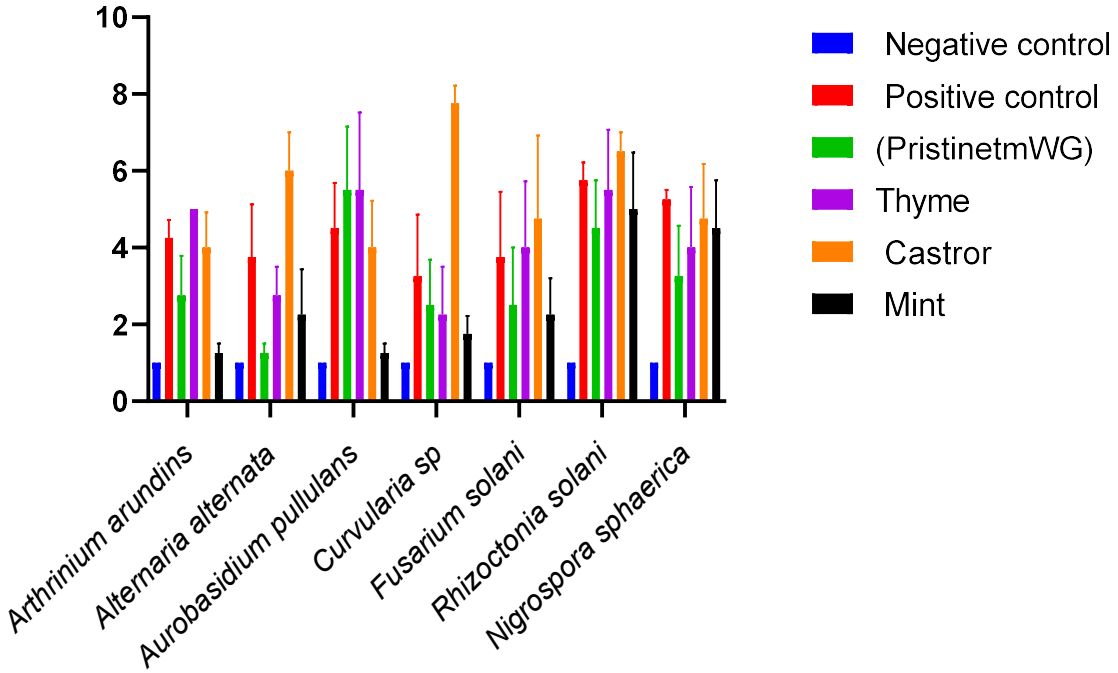

Fig.2 The effect of plant extract (Thyme , castore , Mint) and fungicide (Pristine ^{tm} Wg) for each of the tests, the plant extract impact was very significant (P 0.01) for the mean disease severities and standard errors for seedling wheat plants. For each of the six experiments, the standard error of the means (SEM) is computed.

The percentage germination of wheat grains in green house experiments they had significant effect between the pots treated by plant extracts and fungicides with the positive control the plant extract product seed against seed borne pathogens in soil . showed (table.4) the highest percentage of seed germination the pots treated by thyme plant extract had the fungicide 100 % and recorded the lowest percentage of seed germination the mint plant extract treated 55%.

Table.4The effect of plant extract (Thyme , castore , Mint) and fungicide (Pristine ^{tm} Wg)on the percentage of seeds germination wheat cultivar

Fungal isolates	Negative control	Positive control	(Pristine^{tm} WG) 0.25%	Thyme 0.75%	Castor 0.75%	Mint 0.75%
Arthriniumarundins	90^{ab}	75^{ab}	90^{ab}	75^{ab}	90^{ab}	80^{ab}
Alternaria alternata	75^{ab}	75^{ab}	100^{a}	100^{a}	95^{ab}	60^{ab}
Aurobasidium pullulans	75^{ab}	95^{ab}	100^{a}	70^{ab}	95^{ab}	55^{b}
*Curvularia*sp	80^{ab}	85^{ab}	95^{ab}	75^{ab}	90^{ab}	90^{ab}
Fusarium solani	75^{ab}	85^{ab}	100^{a}	100^{a}	95^{ab}	80^{ab}
Rhizoctonia solani	85^{ab}	70^{ab}	75^{ab}	90^{ab}	90^{ab}	75^{ab}
Nigrosporasphaerica	75^{ab}	85^{ab}	100^{a}	95^{ab}	85^{ab}	70^{ab}

Means had a common letter in the columns are non-significant different at $p \leq 0.01$ as analyzed by Duncan

The maximum shoot lengths and root were obtained in castor 24.87cm shot length in treatment inoculated by *Rhizoctonia solani* root length treatment 14.50cm in negative control treatment ,. minimum shoot lengths by castor treatment 14.87 cm in castor inoculated by *Curvularia* sp and root length 7.37 cm in mint treated was inoculated by *Aurobasidium pullulans*and in positive control treatment were inoculated by *Aurobasidiumpullulans*and*Nigrosporasphaerica* .(Table 5 ,6)

Table.5The effect of plant extract (Thyme , castor , Mint) and fungicide (Pristine ^{tm} Wg)on wheat cultivar shows Foliar length(cm)of seedlings

Fungal isolates	Negative control	Positive control	(Pristine^{tm} WG) 0.25%	Thyme 0.75%	Castor 0.75%	Mint 0.75%
Arthriniumarundins	23.75^{a}	24.50^{a}	23.00^{a}	24.62^{a}	22.25^{ab}	24.12^{a}
Alternaria alternata	23.75^{a}	21.25^{ab}	23.12^{a}	23.62^{a}	21.87^{ab}	22.62^{a}
Aurobasidium pullulans	23.75^{a}	24.25^{a}	23.62^{a}	22.25^{ab}	22.00^{ab}	22.50^{ab}
*Curvularia*sp	23.75^{a}	20.37^{ab}	19.87^{ab}	21.50^{ab}	14.87^{b}	18.62^{ab}
Fusarium solani	23.75^{a}	19.00^{ab}	21.50^{ab}	24.12^{a}	24.12^{a}	23.00^{a}
Rhizoctonia solani	23.75^{a}	20.87^{ab}	23.62^{ab}	23.12^{a}	24.87^{a}	23.12^{a}
Nigrosporasphaerica	23.75^{a}	22.87^{a}	21.75^{ab}	25.37^{a}	20.50^{ab}	20.00^{ab}

Means had a common letter in the columns are non-significant different at $p \leq 0.01$ as analyzed by Duncan

Table .6The effect of plant extract (Thyme , castore , Mint) and fungicide (Pristine ^{tm} Wg) on wheat cultivar shows root length (cm) of seedlings

Fungal isolates	Negative control	Positive control	(Pristine^{tm} WG) 0.25%	Thyme 0.75%	Castor 0.75%	Mint 0.75%
Arthriniumarundins	14.50^{a}	8.75^{ab}	10.50^{ab}	9.62^{ab}	10.00^{ab}	9.62^{ab}
Alternaria alternata	14.50^{a}	8.12^{ab}	9.25^{ab}	10.37^{ab}	12.50^{ab}	12.50^{ab}
Aurobasidium pullulans	14.50^{a}	7.62^{b}	10.25^{ab}	9.50^{ab}	10.75^{ab}	7.37^{b}
*Curvularia*sp	14.50^{a}	8.00^{ab}	11.12^{ab}	9.62^{ab}	10.12^{ab}	8.00^{ab}
Fusarium solani	14.50^{a}	8.00^{ab}	8.25^{ab}	10.75^{ab}	11.12^{ab}	11.00^{ab}
Rhizoctonia solani	14.50^{a}	8.00^{ab}	8.12^{ab}	9.62^{ab}	10.50^{ab}	9.75^{ab}
Nigrosporasphaerica	14.50^{a}	7.37^{b}	7.50^{b}	8.50^{ab}	10.37^{ab}	11.37^{ab}

Means had a common letter in the columns are non-significant different at $p \leq 0.01$ as analyzed by Duncan

Table (7,8) showed the high fresh weight of Pristine^{tm} WG) 1.40 gm inoculated by *Curvularia* sp and root weight 0.33 gm in Pristine^{tm} WG) treated inoculated by Fusarium solani and low weight of length 0.79gm of mint treated , inoculated by *Alternaria alternata* and root weight 0.02 gm in thyme treated inoculated by *Fusarium solani*.

Table.7 The effect of plant (Thyme ,castore , Mint) and fungicide (Pristine ^{tm} WG)on wheat cultivar shows Fresh weight of seedlings

Fungal isolates	Negative control	Positive control	(Pristine^{tm} WG) 0.25%	Thyme 0.75%	Castor 0.75%	Mint 0.75%
Arthriniumarundins	1.16^{ab}	1.15^{ab}	1.31^{a}	1.31^{a}	1.24^{ab}	0.93^{bc}
Alternaria alternata	1.16^{ab}	1.21^{ab}	1.30^{a}	1.24^{ab}	1.25^{ab}	0.79^{c}
Aurobasidium pullulans	1.16^{ab}	1.15^{ab}	1.29^{ab}	1.25^{ab}	1.35^{a}	1.25^{ab}
*Curvularia*sp	1.16^{ab}	1.21^{ab}	1.40^{a}	1.28^{ab}	1.23^{ab}	1.23^{ab}
Fusarium solani	1.16^{ab}	1.21^{ab}	1.16^{ab}	1.30^{a}	1.30^{ab}	1.32^{a}
Rhizoctonia solani	1.16^{ab}	1.18^{ab}	1.31^{a}	1.33^{a}	1.27^{ab}	1.17^{ab}
Nigrosporasphaerica	1.16^{ab}	1.17^{ab}	1.32^{a}	1.30^{a}	1.25^{ab}	1.26^{ab}

Means had a common letter in the columns are non-significant different at $p \leq 0.01$ as analyzed by Duncan

Table.8The effect of plant extract (Thyme , castore , Mint) and fungicide (Pristine ^{tm} Wg) on wheat cultivar shows dry weight of seedlings

Fungal isolates	Negative control	Positive control	(Pristine^{tm} WG)0.25%	Thyme 0.75%	Castor 0.75%	Mint 0.75%
Arthriniumarundins	0.12^{bc}	0.16^{bc}	0.08^{bc}	0.10^{bc}	0.08^{bc}	0.15^{bc}
Alternaria alternata	0.12^{bc}	0.11^{bc}	0.45^{bc}	0.90^{bc}	0.12^{bc}	0.13^{bc}
Aurobasidium pullulans	0.12^{bc}	0.18^{b}	0.47^{bc}	0.09^{bc}	0.06^{bc}	0.10^{bc}
*Curvularia*sp	0.12^{bc}	0.09^{bc}	0.11^{bc}	0.08^{bc}	0.08^{bc}	0.09^{bc}
Fusarium solani	0.12^{bc}	0.07^{bc}	0.33^{a}	0.02^{c}	0.06^{bc}	0.11^{bc}
Rhizoctonia solani	0.12^{bc}	0.12^{bc}	0.07^{bc}	0.03^{c}	0.14^{bc}	0.11^{bc}
Nigrosporasphaerica	0.12^{bc}	0.14^{bc}	0.42^{bc}	0.07^{bc}	0.08^{bc}	0.11^{bc}

Means had a common letter in the columns are non-significant different at $p \leq 0.01$ as analyzed by Duncan

The results revealed that thyme , castor and mint were as effective as the (Pristine^{tm} WG) fungicide, in which they retained the lowest diseases severity index with no significant differences between them.Numerous studies have shown the value of plant extracts in preventing the spread of wheat grain-infecting fungus (Hasan et al., 2005;Shafique et al., 2007;Perelló et al., 2013). These findings are in line with those of other researchers who claimed that plant extracts may be utilized to improve wheat seed germination and seedling vigor (Perelló et al., 2013, Hasan et al., 2005, Shafique et al., 2007). The inhibition of the incidence of the seed-borne fungus that may have destroyed the grain embryo might be responsible for the extracts' capacity to promote seedling emergence and grain germination.

4. Conclusions

plant extracts as bio fungicides, may work efficiently against a range of fungal wheat seed borne pathogens including ((*Aurobasidiumpullulans , Curvulariasp , Fusarium solani , Alternaria aalternata, Arthriniumarundinis ,Rhizoctonia solani, Nigrosporaspaerica*.) .

laboratory experiment using agar plate method, plant extracts tested in poison food technique they have significant effect between treatments thyme plant extract had highest effect of inhabitation of mycelial growth of tested fungi. Affect plant extract on the post emergence damping off they significant effect between treatments all plant extract decrease number off seedling had post emergence damping off. Mint plant extract had high effect to reduce diseases severity. Highest number germination wheat seed in the pots treated by thyme plant extract. Three plant extract had significant effect on the shoot length and they had lowest effect on the root length comparison with negative control. They had significant effect of fresh and dry seedling wheat plant between all treatments comparison with negative control.

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