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In vitro Evaluation of Fungicide and Bio-Pesticides against Isolates of *Alternaria alternata* (Fr.) Black Spot

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A B S T R A C T

The efficacy of seven fungicides and seven bio pesticides were evaluated *invitro* against isolates of *A. alternata*, among the different fungicides tested, the maximum inhibition was recorded by hexaconazole (0.1%) with of 97.84 per cent inhibition, followed by propiconazole (96.31%) and tebuconazole (94.45%) and the least inhibition was recorded by carbenazime with 40.94 per cent. Among different isolates the 100 per cent inhibition was recorded by many isolates against triazole fungicides and the least inhibition was recorded by isolate Aa353 on Chlorothalonil (0.2%). Among different bio pesticides tested the maximum inhibition per cent was noticed in Garlic bulb extract with mean per cent inhibition of 54.24 per cent followed by Neem leaf extract (48.92%), whey (48.11 %) and cow urine (45.62 %). The lowest inhibition was recorded by desi cow milk with mean inhibition of 36.26 per cent. Among different isolates Aa1728 (69.63%) showed maximum inhibition by neem leaf extract and least was recorded by Aa 1729 (13.71%) on desi cow milk.

Introduction

Pomegranate (*Punica granatum*) is grown in tropical and subtropical regions of the world. The native range of pomegranate (*Punica granatum* L.) spans from Iran to the Himalayas in northern India, and this crop has been cultivated throughout the Mediterranean region since ancient times. Due to increasing evidence for health-promoting effects of pomegranate, the consumption of pomegranate fruit and juice has been increasing, as well as the amount of area dedicated to its cultivation.

At present, Maharashtra with an area of 136.75 thousand ha is the leading state in acreage and accounts for 68.7 per cent of the total area under pomegranate in the country. Other major pomegranate growing states are Karnataka (28.08 thousand ha) with production (328.92 thousand MT) having productivity of 11.71 MT per hectare (2016-17). Gujarat is in third position 18.54 thousand

ha cultivation of pomegranate with production of 278.10 thousand MT, having productivity of 15 MT per ha and Andhra Pradesh (7.71 thousand ha). In recent years, pomegranate cultivation has also been started in Rajasthan, Orissa, Chhattisgarh, Uttarakhand and Madhya Pradesh at small scale (Anon, 2017).

The most popular varieties suitable for processing and table purposes are Ganesh, Mridula, Arakta, Bhagwa (Kesar), G-137 and Khandar in India. Though bacterial blight infection on pomegranate due to *Xanthomonas axonopodis* pv. *punicae* has attracted attention by researchers, growers and policy makers alike. Nevertheless, the infections due to fungal species which cause diseases such as anthracnose (*Colletotrichum gloeosporioides*), leaf spot and severe fruit spot/rot by *Alternaria alternata*, *Cercospora* spp., *Drechslera* spp. and *Sphaceloma* spp. *etc.* are more or less equally important and harmful in some orchards. Among these; severe spotting and fruit rotting due to *Alternaria alternata*; remainshitherto unexplored but potentially dangerous pathogen on pomegranate and considered tobe an emerging disease. *Alternaria alternata* (Fr.) Keissler is a ubiquitous necrotrophic fungus.

The novel symptoms of black spot of pomegranate caused by *A. alternata* in pomegranate orchards recently have reported in Israel. These symptoms include black spots on leaves and fruit, ranging from a single lesion to lesions that cover more than 50% of the fruit surface, as well as chlorosis and the abscission of some leaves. Lesions begin to appear on all cultivars at the beginning of summer which is round on fruit and round to irregular on leaves. They are surrounded by a green-yellow halo. On fruit, the damage is restricted to the peel surface while the edible tissue remains unaffected. This is in contrast to black rot of pomegranate, in which the fruit rot is restricted to the internal area whereas the peel and leaves remains unaffected. Thus, *A. alternata* that causes black spot of pomegranate is different from the one that causes internal rot of pomegranate.

There is little information available on management of *Alternaria* disease on pomegranate; many fungicides/chemicals available in the market are being sprayed against various fungal foliar infections seldom achieving the desire result.

Hence *in vitro* studies on bio efficacy and suitability for their application in field conditions need careful investigations. Keeping in view the importance of the disease, the present study was conducted 2016-18 to the efficacy of different fungicides and bio pesticides *in vitro* against *A. alternata.*

Materials and Methods

Alternaria alternata was isolated from the infected leaves of pomegranate collected from Indian peninsular region. After performing their pathogen city test their culture was maintained on Potato dextrose agar medium at 27± 1^0C.

In vitro evaluation of fungicides against *A. alternata*

The efficacy of fungicides was tested against *A. alternata* for radial growth inhibition on the Potato dextrose agar medium using poisoned food technique under *in vitro* condition. Twenty ml of poisoned medium was poured in each sterilized Petriplates. Suitable check was maintained without addition of fungicide. Mycelial disc of 5 mm taken from the periphery of 12 days old colony was placed in the centre of Petri platesand incubated at 27 ±1 ^{0}C for 12 days and three replications were maintained for each treatment. The diameter of the colony was measured in two directions and average was recorded. Per cent inhibition

of mycelial growth of the fungus was calculated by using the formula by Vincent (1947).

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

Where,

I = Per cent inhibition
C = Radial growth in control
T = Radial growth in treatment (fungicide)

Evaluation of various bio pesticides against *A. alternata*

Preparation of cold aqueous extract

Fresh plant materials were collected and washed first in tap water and then with distilled water. Fresh samples (100 g) of different botanicals were chopped and then crushed in a surface sterilized pestle and mortar by adding 100 ml sterile distilled water (1:1 w/v). The extract was filtered through two layers of muslin cloth and finally filtrate thus obtained was used as stock solution. To study the antifungal mechanism of plant extracts the poisoned food technique was adopted.

Twenty ml of such medium was poured under aseptic conditions into sterile Petri plates allowed to solidify. Mycelial discs (5mm) were cut out using sterile cork borer from periphery of actively growing culture and one such disc was placed on the centre of each Petri plate. The treatments were replicated thrice. Control was maintained by growing the pathogen on PDA plates without poisoning with plant extract. Plates were incubated at room temperature (28±1°C) for 12 days. The diameter of the colony was measured in two directions and average was recorded. Per cent inhibition of mycelial growth of the fungus was calculated by using the formula by Vincent (1947).

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

Where,

I = Per cent inhibition
C = Radial growth in control
T = Radial growth in treatment (fungicide)

Results and Discussion

In vitro evaluation of fungicides

Among the different fungicides tested the maximum of 100 per cent inhibition was found in Hexaconazole (97.84%), followed by Propiconazole (96.31%) and tebuconazole (94.45) and the least inhibition was recorded with the carbenazime with 40.94 per cent succeeded by Chlorothalonil (46.47%) and thiophanate metyle (52.49%) of mycelial inhibition. Among different isolate tested in respect to Mancozeb the highest per cent inhibition was recorded with isolate Aa1727 (85.93 %), followed by Aa368 (85.19 %), Aa3511 (84.07%) and Aa361 (82.69 %) shows significantly superior over the other isolates and the least inhibition of 47.78 per cent was recorded with the isolate Aa353 (Table.2.1). On Thiophanate methyle treated media the maximum mycelial inhibition of 77.78 per cent was found with isolate Aa2417 followed by isolates Aa368 and Aa2416 with inhibition of 73.19 and 72.59 respectively, the lowest inhibition was shown by isolate Aa2523 (31.22 %) (Table.2.2). On Chlorothalonil the maximum inhibition of 67.19 per cent was noticed in isolate Aa1727, which was significantly superior over all other treatments. Significantly least per cent inhibition was recorded in isolateAa2318 (30.00 %).On Hexaconazole the maximum inhibition of 100 percent was recorded with many isolates and the least per cent inhibition of 94.44 per cent was reported in isolate Aa2523.On Propiconazole also 100 percent was recorded

with many isolates such as Aa362, Aa358, Aa2318, Aa2322, Aa1725 and Aa1729. The lowest92.04 per cent inhibition was reported in isolate Aa369.On Carbendazime the lowest inhibition of 25.19 per cent was noticed in isolate Aa2420 which was on par with the isolates Aa2522 (27.04%) Aa2318 (27.26%) and Aa353 (28.19%), the highest mycelial inhibition 62.96 per cent was recorded by the isolates Aa1727 and Aa3610.

Tebuconazole was also very effective against the isolates tested, with 100 per cent inhibition shown in many isolate such as Aa368, Aa369, Aa3511, Aa2319, Aa2522 and Aa1725 and the least per cent inhibition of 90.37 per cent was reported in isolate Aa1730. Similar results were obtained by the author Mahantesh *et al.*, (2017) who reported that the contact fungicide Mancozeb at 1000 ppm, systemic fungicide, Hexaconazole at 1000 ppm were effective against *A. solani* causing early blight in tomato.

These results are also in conformity with the author Vasudha *et al.*, (2018) who found that average cent per cent inhibition of mycelial growth was with Propiconazole followed by Hexaconazole, Penconazole and Difenconazole.

Mallikarjun (1996) recorded in vitro evaluation of eight fungicides against *A. alternata* causing leaf blight of turmeric; where in propiconazole (Tilt) was found to be superior in inhibiting the growth of the fungus.

Table.1 Fungicides treatment details

Tr. No.	Fungicides name	Trade name	Concentrations (%)
T1	Mancozeb	Indofil M-45 75% WP	0.2
T2	Thiophanate methyl	Roko 70% WP	0.2
T3	Chlorothalonil	Kavach 75% WP	0.2
T4	Hexaconazole	Contaf 5% EC	0.1
T5	Propicanazole	Tilt 25% EC	0.1
T6	Carbendazim	Bavistin 50% WP	0.2
T7	Tebuconazole	Folicur 25% EC	0.1
T8	Control		

Table.2 Treatment details bio pesticides used

Sl. no	Bio pesticides name	Scientific name	Plant Part used	Concentration (%)
1	Garlic bulb extract	*Allium sativum*	Bulb	10
2	Neem leaf etract	*Azadirachta indica*	Leaf	10
3	Durantha leaf extract	*Duranta erecta*	Leaf	10
4	Karanj leaf etract	*Pongamia pinnata*	Leaf	10
5	Whey	Gir	-	10
6	Milk	Gir	-	10
7	Cow urin	Gir	-	10
8	Control	-	-	

Table.2.1 *In vitro* evaluation of fungicides against isolates of *A. alternata*

Isolates	Per cent inhibition of mycelial growth overcontrol						
	Mancozeb (0.2%)	Chlorothalonil (0.2%)	Thiophanate methyle (0.2%)	Hexaconazole (0.1%)	Propiconazole (0.1%)	Carbendazime (0.2%)	Tebuconazole (0.1%)
Aa 361	82.96	61.48	56.67	96.30	97.78	61.48	91.11
Aa 362	71.85	30.37	62.59	100.00	100.00	54.07	93.70
Aa 353	47.78	12.22	33.70	96.30	95.00	37.41	92.59
Aa 364	75.56	31.48	41.85	95.19	95.19	35.19	91.11
Aa 365	80.00	20.00	51.48	98.15	95.37	53.70	91.48
Aa 356	74.81	17.04	45.56	100.00	98.15	51.85	94.07
Aa 367	66.67	51.85	35.56	100.00	95.56	41.85	94.44
Aa 368	85.19	30.37	75.19	97.41	100.00	43.70	100.00
Aa 369	71.11	64.07	64.81	94.81	92.04	49.26	100.00
Aa 3610	78.15	43.70	40.00	100.00	100.00	62.96	93.70
Aa 3511	84.07	26.67	63.70	95.93	95.07	38.52	100.00
Aa 3612	55.19	30.37	53.70	95.93	92.48	41.11	91.11
Aa 3613	72.96	17.04	50.37	98.15	95.93	50.74	91.11
Aa 2514	79.63	51.11	49.63	100.00	96.30	35.19	92.96
Aa 2415	74.07	41.48	71.85	97.78	94.44	51.48	93.33
Aa 2416	82.96	58.52	72.59	100.00	92.22	44.81	98.15
Aa 2417	77.04	45.93	77.78	97.78	96.11	47.41	92.22
Aa 2318	72.22	19.26	60.74	100.00	100.00	30.00	94.07
Aa 2319	71.85	47.41	55.56	95.56	95.56	48.89	100.00
Aa 2420	74.07	34.44	54.44	100.00	93.22	25.19	100.00
Aa 2421	84.07	38.52	34.81	100.00	93.52	37.04	92.96
Aa 2522	64.44	33.33	35.93	100.00	100.00	35.19	100.00
Aa 2523	77.78	21.48	25.19	94.44	92.22	52.59	94.07
Aa 1724	74.07	40.74	44.44	97.41	98.15	51.85	94.07
Aa 1725	78.52	44.44	41.85	95.93	100.00	50.74	93.33
Aa 1726	72.22	44.07	50.37	100.00	93.56	46.30	91.85
Aa 1727	85.93	65.19	57.04	100.00	100.00	62.96	97.78
Aa 1728	76.30	60.37	56.30	94.81	98.15	50.37	91.85
Aa 1729	72.22	58.89	55.93	95.56	100.00	28.52	92.22
Aa 1730	71.11	56.30	54.07	97.78	93.33	49.63	90.37
CD@5%	4.66	4.76	3.95	5.28	4.13	4.58	2.09
S.Em	1.65	1.68	1.40	1.87	1.46	1.62	0.74

Table.2.2 *In vitro* evaluation of bio-pesticides against isolates of *A. alternata*

Isolates	Per cent Inhibition of mycelial growth over controle (mm)						
	Neem leaf	Garlic	Duranta	Karanj	Cow milk	Cow urine	Whey
Aa361	28.15	54.81	30.74	17.04	25.93	59.26	49.63
Aa362	49.63	28.52	37.41	43.70	32.59	45.19	55.56
Aa353	47.41	63.33	28.89	38.89	40.74	59.26	51.11
Aa364	61.48	67.04	50.37	56.30	43.33	39.63	54.44
Aa365	49.26	40.74	55.19	53.70	30	46.3	55.19
Aa356	59.63	50.00	32.22	47.04	25.56	26.3	31.48
Aa367	33.7	50.00	41.11	40.74	27.78	24.07	41.48
Aa368	42.96	61.11	28.89	30.00	22.22	60.00	28.89
Aa369	58.15	60.37	36.30	53.70	54.07	63.70	55.56
Aa3610	43.33	62.59	45.56	60.00	21.48	41.48	59.26
Aa3511	62.22	48.89	20.37	39.26	45.19	55.93	63.70
Aa3612	66.30	37.04	35.56	26.30	40	31.11	50.37
Aa3613	44.44	30.74	42.96	24.44	52.96	60.74	57.78
Aa2514	58.52	33.7	35.19	41.11	47.41	51.11	47.78
Aa2415	56.30	35.56	43.70	47.41	34.44	55.93	49.26
Aa2416	58.89	37.78	35.19	62.96	40.37	41.85	46.3
Aa2417	61.48	60	42.59	33.33	37.78	57.04	42.59
Aa2318	21.48	43.33	31.48	13.33	28.89	44.07	37.04
Aa2319	23.33	69.63	25.19	49.26	30.37	61.85	47.41
Aa2420	68.15	47.04	37.78	68.15	34.44	65.56	62.22
Aa2421	57.41	62.59	41.11	60.37	27.78	53.7	50.37
Aa2522	49.63	65.56	27.41	65.56	31.48	42.59	50.74
Aa2523	62.59	31.85	46.00	24.44	22.96	34.81	51.11
Aa1724	39.63	59.26	31.85	57.41	48.15	38.52	31.85
Aa1725	34.44	55.19	13.70	59.26	37.78	54.44	40.00
Aa1726	65.93	30.74	32.22	30.00	21.85	28.89	25.19
Aa1727	49.26	57.78	45.93	42.59	42.59	31.48	51.11
Aa1728	69.63	60.74	57.78	64.44	57.78	41.48	57.78
Aa1729	61.48	62.59	28.89	21.11	13.7	28.52	66.30
Aa1730	28.89	52.96	53.70	16.30	52.59	24.07	31.85
CD@5%	6.14	5.03	5.02	5.34	4.26	5.25	5.47
S.Em	2.17	1.78	1.77	1.89	1.51	1.86	2.37

Evaluation of bio-pesticides

Among different bio pesticides tested the maximum inhibition per cent was noticed in Garlic bulb extract with mean per cent inhibition of 54.24 per cent followed by Neem leaf extract (48.92%), whey (48.11 %) and cow urine (45.62 %). The lowest inhibition was recorded by desi cow milk with mean inhibition of 36.26 per cent

Among different isolate tested on Neem leaf extract the highest per cent inhibition was recorded with isolate Aa1728 (69.63%) followed by Aa3612 (66.30%), Aa1726 (65.93 %), and Aa3511 (62.22%) shows significantly superior over the other isolates and the least inhibition of 21.48 per cent was recorded with the isolate Aa2318.The fungicidal spectrum of *Azadirachta indica* has been attributed to azadiractin which belongs to C25 terpenoides (Subramaniam, 1993). On Duranta leaf extract, the maximum mycelial inhibition of 57.78 per cent was found with isolate Aa1728 followed by isolates Aa365 with inhibition of 55.19 per cent, the lowest inhibition was shown by isolate Aa3511 (20.37 per cent). On Cow milk the maximum inhibition of 60.00 per cent was noticed in isolate Aa353 followed by Aa1728 (57.78), Aa369 (54.07%) and Aa3613 (52.96%) which was significantly superior over all other treatments. the least per cent inhibition was recorded in isolate Aa 1729(13.71%). On karanj leaf extract the highest mycelial inhibition 65.56 per cent of was recorded by the isolates Aa2522 followed by isolate Aa1728 (64.44%), Aa2415 (62.96%), Aa2421 (60.37%) and the lowest inhibition of 13.33 per cent was noticed in isolate Aa2318. Garlic bulb extract is also very effective against the isolates tested 10 per cent concentration, among the isolates Aa1724 (69.63%) followed by Aa361 (67.04%) and Aa1729 (62.59%), the least per cent inhibition of 28.52 per cent was reported in isolate Aa353.The antifungal action of garlic is due to the compound allicin. It has strong antimicrobial and antifungal activities (Abdulaziz *et al.*, 2018). On cow urine, the maximum inhibition of 65.56 per cent was noticed in isolate Aa2420 followed by Aa369 (63.70%) and Aa3613 (52.96%) which was significantly superior over all other treatments. the least per cent inhibition was recorded in isolate Aa1730 (13.70%).

Antimicrobial activity of camel urine is due to factors such as high salt concentrations, alkalinity, and natural bioactive compounds (Kamlu *et al.*, 2004).Presence of urea, creatinine, swarn kshar (aurum hydroxide), carbolic acid, phenols, calcium, and manganese has strongly explained the antimicrobial and germicidal properties of CU (Achliya *et al.*, 2004; Jain *et al.*, 2010; Kumar, 2001). On whey the highest mycelial inhibition 66.30per cent of was recorded by the isolates Aa1729 followed by isolate Aa3517 (63.70%), Aa2420 (62.22%) and the lowest inhibition of 21.11 per cent was noticed by isolate Aa1729.

Results are in conformity with the author (Peter *et al.*, 2006) reported that milk and whey caused the hyphae of *E. necator* to collapse and damaged conidia within 24 h of treatment is due to lactoferrin (An antimicrobial component of milk). Vaibhav *et al.*, (2018) reported that among different plant extracts used against *A. solani*, showed that *Azadirachta indica* (Neem) was significantly inhibit the mycelial growth of pathogen at all concentrations followed by *Datura strumarium* (Jimson weed) and *Calotropis gigantea* (Aak).

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