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In vitro screening of fungicides and tannins against fungal pathogens of jujube fruits

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Abstract

The aim of this study to identify the effective fungicides for controlling major leaf and fruit diseases of Jujube caused by different fungal pathogens. An *in vitro* experiment was undertaken to screen ten fungicides and two Tannins against six important fungal pathogens of Jujube fruits which were *Alternaria alternata, Colletotrichum gloeosporioides, Curvularia lunata, Lasiodiplodia theobromae, Fusarium semitectum and Pestalotiopsis palmarum*. Fungicides were Conza 5 EC (Hexaconazole), Folicur 250 EC (Tebuconazole) and Potent 250 EC (Propiconazole), Bavistin DF (Carbendazim), Kasumin 2% liquid (Kasugamycin), Rovral 50 WP (Iprodine), Matco 72 WP (Mancozeb 64% + Metalaxyl 8%), Geneb 80 WP (Mancozeb), Emivit 50 WP (Copper oxychloride) and Evavit 80 WG (Sulphur 80 WG). And two tannins were Chestnut Tannin and Quabracho Tannin. Plain water was used as control. Poison food technique was followed to conduct the experiment. Both Tannins did not show satisfactory inhibition of mycelial growth of any of the six fungal pathogens. The effectiveness of fungicides varied greatly with fungal species. The most effective fungicides were Folicur 250 EC followed by Potent 250 EC, Rovral 50 WP, Conza 5 WP and Bavistin DF. Folicur 250 EC caused 89.52% inhibition of mycelium growth of *A. alternata* and 100% growth inhibition in other five fungi. In addition, Potent 250 EC inhibited growth of *L. theobromae, C. lunata, F. semitectum* and *P. palmarum* by 100% and that of *C. gloeosporioides* by 91.79%. Complete growth inhibition of *F. semitectum* and *P. palmarum* was achieved with Bavistin DF. Furthermore, Conza 5 EC caused 100% growth inhibition in *C. lunata* and more than 90% in *L. theobromae* and *F. semitectum.*

Key words: Screening, fungicides, tannins, fungal pathogens, jujube

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Introduction

Jujube (*Ziziphus mauritiana* Lam.) tree is subjected to attack by different fungal pathogens causing various diseases. In India, powdery mildew *(Oidium* sp.*)* causes defoliation and fruit-drop. Sooty mold *(Cladosporium zizyphi)* causes leaves to fall. Leaf spot results from infection by *Cercospora* spp. and *Isariopsis indica* var. *zizyphi.* Leaf rust, caused by *Phakopsora zizyphi-vulgaris,* ranges from mild to severe on all commercial cultivars in India. Fruits on the tree are attacked by *Alternaria chartarum, Aspergillus nanus, A. parasiticus, Helminthosporium atroolivaceum, Phoma hessarensis,* and *Stemphyliom mavalparadisiacum.* Twigs and branches may be affected by *Entypella zizyphi, Hypoxylon hypomiltum,* and *Patellaria atrata.* In storage, the fruits may be spotted by the fungi *Alternaria brassicicola, Phoma* spp., *Curvularia lunata, Cladosporium herbarum.* Fruit rots are caused by *Fusarium* spp., *Nigrospora oryzae, Epicoccum nigrum,* and *Glomerella cingulata* (Morton, 1987). Reports from different countries reveal that the crop is attacked by powdery mildew (*Oidium erysiphoides* f. sp. *ziziphi*), fruit spot and rot (*Colletotrichum gloeosporioides*, *Alternaria alternata*, *Fusarium* spp., *Pestalotiopsis palmarum*, *Curvularia lunata*, *Lasiodiplodia theobromae*, *Aspergillus* spp.), sooty mould, leaf rust, leaf spot etc. (Gupta and Madan 1977a; 1977b; 1977c; Rai*et al.,* 1982; Singh and

Sumbali, 2000; Chang, 2004; Jamadar*et al.,* 2009; Yuan *et al.,* 2009).

Anthracnose disease is a worldwide problem owing to the severe economic damage to tropical fruits including Jujube in Taiwan (Chung *et al*., 2010). It is an important disease of Chinese Jujube that mainly attacks 1 to 2 year-old branches and can damage 16-20% of fruits (sometimes up to 50%) (Chang, 2004). *Alternaria alternata* causes pre-harvest infection on Jujube fruits that results 25% yield loss (Nallathambi, 2001; Nallathambi*et al.,* 2006). Moreover, as because of perishable in nature Jujube fruits are highly susceptible to post harvest colour fading, browning and vulnerable to post harvest losses due to fungal diseases (Tian*et al.,* 2005).
Crop loss due to Jujube diseases in Bangladesh has not yet been estimated. But some field observations revealed that powdery mildew (*Oidium erysiphoides* f. sp. *ziziphi*), anthracnose (*C. gloeosporioides*) and fruit spot or rot (caused by *A. alternata*, *Fusarium* spp., *Pestalotiopsis palmarum*, *Curvularia lunata*, *Lasiodiplodia theobromae*) were severe problem of Jujube cultivation in Bangladesh. Major Pre and post harvest losses were caused by these diseased.

Several researchers of different countries tested fungicides to control fungal pathogens causing fruit diseases (Lei *et al.,* 2000; Hu *et al.,* 2002; Chang, 2004; Qin and Tian, 2004; Li *et al.,* 2005; Chung *et al*., 2010). In Bangladesh, such types of researches on the management of pre and post-harvest diseases of Jujube fruits are not available. Therefore, the present investigation was undertaken to screen fungicides and tannins against six important fungal pathogens which are mostly responsible for pre and post-harvest diseases of Jujube fruits with a view to find out effective methods to control six pathogens.

Materials and methods

Ten different fungicides and two tannins were tested in the present experiment under in vitro condition against six major fungal fruit pathogens of Jujube namely *A. alternata, L. theobrome, C. gloeosporioides, C. lunata, F. semitectum* and *P. palmarum*. Fungicides Conza 5 EC (Hexaconazole),Folicur 250 EC (Tebuconazole) andPotent 250 EC (Propiconazole) were used @ 0.05%. Bavistin DF (Carbendazim), Kasumin 2% liquid (Kasugamycin), Rovral 50 WP (Iprodine), Matco 72 WP (Mancozeb 64% + Metalaxyl 8%) andGeneb 80 WP (Mancozeb) were applied @ 0.2%. Emivit 50 WP (Copper oxychloride), Evavit 80 WG (Sulphur 80 WG), Chestnut Tannin and Quabracho Tannin were used @ 0.3%.For comparison, a control treatment was maintained in each experiment.

Poison food technique was followed and potato dextrose agar (PDA) was used as the basal medium (Tuite 1969). After sterilization, 20 ml melted PDA was poured into sterilized Petri dishes and required quantity of each fungicide or tannin was added to the medium and mixed thoroughly. The amended PDA was allowed to solidify and inoculated with 6 mm mycelial discs of previously grown PDA culture of individual test fungi. After inoculation all plates were wrapped with Para film for sealing and placed in an incubator in inverted condition at 28±1 ^{0} C for 12 days. The plates contain only PDA media without any fungicides or tannin, served as control.

Separate experiment was conducted for each fungus. Each of the experiments was conducted following a completely randomized design with four replications (Petri dishes). At 1, 2, 5, 8 and 12 days after incubation (DAI), data on radial diameter of mycelium growth were measured. Final colony growth data were collected at 8 DAI for all tested fungi except *L. theobromae* and *P. palmarum*. Data of *L. theobromae* and *P. palmarum* were collected at 2 and 12 DAI, respectively. For measurement, two lines were drawn at right angle on the back side of the plates with a marker pen so that their intersection sets on the central point of the fungal disc. The radial growth rate, per cent radial growth inhibition and change of mycelial colour as compared to control were observed and recorded. Per cent radial growth inhibition over control was calculated by using the formula suggested by Vincent (1947).

$$Percentinhibition = \frac{(Radial growth in control plate) - (Radial growth on amended PDA)}{Radial growth in control plate} x100$$

Finally data were analyzed statistically using MSTAT-C and means were compared following Turkey's Test.

Results and Discussion

Radial growth of colony

Alternaria alternata: Except Q Tannin, amendment of PDA with eight fungicides and C Tannin reduced radial colony diameter of *A. alternata* significantly compared to control (Table 1). The reduction under different treatments ranged 6.07-89.52%. The highest reduction was achieved with Folicur 250 EC followed by Potent 250 EC. Their efficacy was statistically similar but significantly higher compared to other treatments. Conza 5 EC, Rovral 50 WP and Emivit 50 WP gave 76.83, 72.38 and 65.20% reduction in colony growth of *A. alternata* in-vitro. Other treatments caused only 6.07-29.09% reduction in its growth. The reduction under C Tannin, Q Tannin and Evavit 80 WG was statistically similar and significantly lower compared to all other treatments (Table 2 and Figure 1).

Lasiodiplodia theobromae: In-vitro colony growth of *L. theobromae* was reduced significantly over control due to amendment of PDA with all fungicides except Kasumin 2% liquid, and two Tannins (Table 1). The growth inhibition was 100% over control under Rovral 50 WP, Folicur and Potent 250 EC.Its growth inhibition over control was 94.44, 94.05, 97.26 and 99.21% due to amendment of PDA with Conza 5 EC, Bavistin DF, Matco 70 WP and Emivit 50 WP, respectively. Less than 40% growth inhibition was found under Geneb 80 WP and Evavit 80 WG and Kasumin 2% liquid showed only 1.31% growth inhibition of the fungus (Table 2 and Figure 1).

***Colletotrichum gloeosporioides*:** Except Kasumin 2% liquid, Evavit 80 WG, C Tannin and Q Tannin, other treatments caused significant reduction of *in-vitro* radial colony diameter of *C. gloeosporioides* compared to control (Table 1). Total growth (100%) inhibition was achieved with only Folicur 250 EC, which statistically similar to Potent 250 EC. Conza 5 EC, Bavistin DF and Rovral 50 WP gave 77.62, 76.71 and 74.57% inhibition over control, respectively (Table 2 and Figure 1).

Cuvularia lunata: In-vitro colony growth of *C. lunata* was reduced significantly over control due to amendment of PDA all fungicides tested in the present experiment. The colony diameter under two Tannins and control was not significantly different (Table 1). Growth of the fungus was completely (100%) inhibited by Conza 5 EC, Rovral 50 WP, Folicur 250 EC and Potent 250 EC. Its growth inhibition was only 2.78-34.52% under other fungicides and two Tannins (Table 2 and Figure 1).

Fusarium semitectum: Radial colony diameter of *F. semitectum* was significantly reduced over control due to amendment of PDA with Conza 5 EC, Bavistin DF, Rovral 50 WP, Folicur 250 EC, Matco 70 WP, Potent 250 EC, Geneb 80 WP and Emivit 50 WP (Table 1). The reduction was 100% under Bavistin DF, Folicur 250 EC and Potent 250 EC.Conza 5 EC, Emivit 50 WP and Geneb 80 WP caused 99.21, 98.41 and 65.20% reduction of radial growth, respectively. Other fungicides and C Tannin gave 1.98-33.89% inhibition of the fungus over control. Q Tannin had not any inhibitory effect on *F. semitectum* (Table 2 and Figure 1).

Pestalotiopsis palmarum: Except two Tannins and Emivit 50 WP, amendment of PDA with other fungicides inhibited colony growth of *P. palmarum* significantly over control (Table 1). Complete growth inhibition of the fungus was achieved with Bavistin DF, Folicur 250 EC and Potent 250 EC. The growth inhibition of the fungus was 95.83, 94.45 and 80.16% over control under the treatments with Matco 70 WP, Geneb 80 WP and Conza 5 EC, respectively. Other materials showed only 2.78-34.40% growth inhibition (Table 2 and Figure 1).

Findings of the present experiment revealed that both C Tannin and Q Tannin did not show satisfactory inhibition of mycelial growth of any of the six fungal pathogens.The fungicides caused variable degree of growth inhibition of the fungal pathogens tested on amended PDA. Their effectiveness varied greatly with fungal species. Folicur 250 EC caused 89.52% inhibition of mycelium growth of *A. alternata* and 100% growth inhibition in other five fungi. Potent 250 EC inhibited growth of *L. theobromae, C. lunata, F. semitectum* and *P. palmarum* by 100% and that of *C. gloeosporioides* by 91.79%. Complete growth inhibition of *F. semitectum* and *P. palmarum* was achieved with Bavistin DF. Conza 5 EC caused 100% inhibition in *C. lunata* and more than 90% in

L. theobromae and *F. semitectum.* The results indicate that the most effective fungicides were Folicur 250 EC followed by Potent 250 EC, Rovral 50 WP, Conza 5 WP and Bavistin DF. They have potentiality to use against the fungal pathogens like *L. theobromae, C. gloeosporioides, C. lunata, F. semitectum, P. palmarum* and *A. alternata* causing fruit rot of Jujube. The findings are in agreement with the findings of other researchers (Anon. 1990, 1997,Talukder*et al*., 2004, Bakr 2007). However, they worked with other crops.

Table 1. Effect of ten fungicides and two tannins on radial colony growth of six fungi causing fruit rot of Jujube

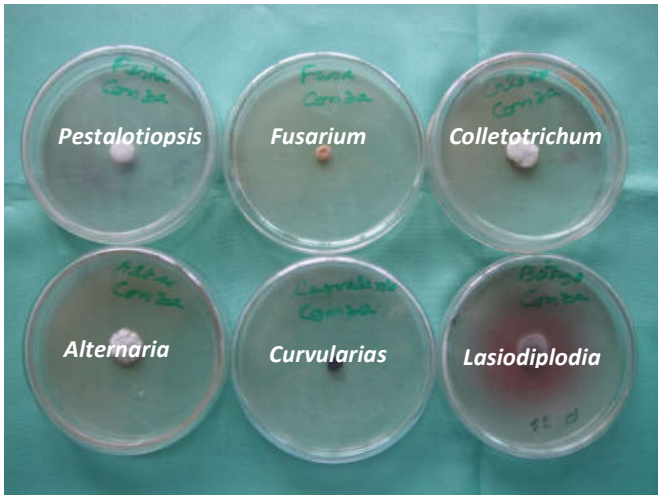
Fungicides	*Alternaria Alternate*	*Lasiodiplodia theobromae*	*Colletotrichum gloeosporioides*	*Curvularia lunata*	*Fusarium semitectum*	*Pestalotiopsis palmarum*
	Radial colony diameter (cm)					
Conza 5 EC	1.95f	0.47d	1.88cd	0.00f	0.07e	1.67d
Kasumin 2% liquid	6.82c	8.29a	7.82a	5.50d	7.90a	5.51c
Bavistin DF	6.72c	0.50d	1.96cd	7.28b	0.00e	0.00f
Rovral 50 WP	2.32f	0.00e	2.14c	0.00f	6.69b	0.83e
Folicur 250 EC	0.88g	0.00e	0.00e	0.00f	0.00e	0.00f
Matco 70 WP	5.96d	0.23de	5.09b	7.20b	5.55c	0.35ef
Potent 250 EC	1.14g	0.00e	0.69de	0.00f	0.00e	0.00f
Geneb 80 WP	6.11d	5.73b	6.30b	7.41b	2.92d	0.47ef
Emivit 50 WP	2.92e	0.07e	5.23b	2.22e	0.13e	8.23ab
Evavit 80 WG	7.55b	5.19c	7.76a	6.23c	8.23a	7.81b
C Tannin	7.50b	8.40a	8.29a	8.17a	7.91a	8.17ab
Q Tannin	7.89ab	8.40a	8.28a	8.08a	8.40a	8.12ab
Control	8.4a	8.40a	8.40a	8.40a	8.40a	8.40a
CV (%)	3.60	3.63	8.91	4.20	8.11	5.02

Means within the same column having a common letter (s) do not differ significantly (P=0.05). DAI means days after incubation

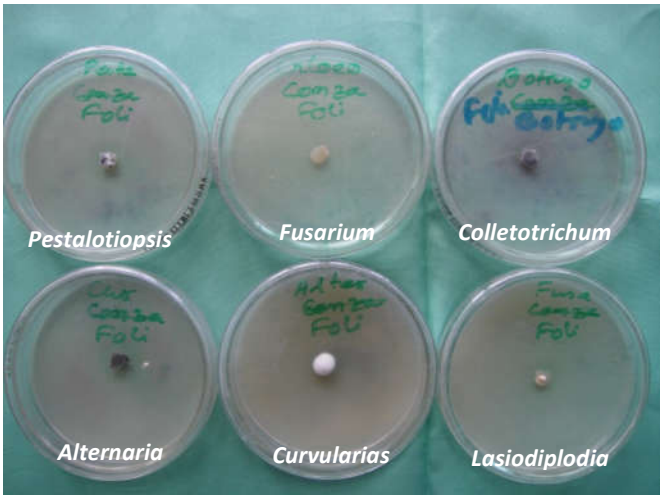
Table 2. Effect of ten fungicides and two Tannins on inhibition of radial colony growth of six fungi causing fruit rot of Jujube

Fungicides	*Alternaria Alternate*	*Lasiodiplodia theobromae*	*Colletotrichum gloeosporioides*	*Curvularia lunata*	*Fusarium semitectum*	*Pestalotiopsis palmarum*
	% Reduction in radial colony diameter over control					
Conza 5 EC	76.83b	94.44bc	77.62bc	100.00a	99.21a	80.16c
Kasumin 2%liquid	18.8e	1.31f	6.91e	34.52c	5.95e	34.40d
Bavistin DF	20.00e	94.05c	76.71bc	13.38e	100.00a	100.00a
Rovral 50 WP	72.38b	100.00a	74.57c	100.00a	20.36d	90.08b
Folicur 250 EC	89.52a	100.00a	100.00a	100.00a	100.00a	100.00a
Matco 70 WP	29.09d	97.26abc	39.44d	14.29e	33.89c	95.83ab
Potent 250 EC	86.39a	100.00a	91.79ab	100.00a	100.00a	100.00a
Geneb 80 WP	27.26d	31.75e	25.00d	11.79e	65.20b	94.45ab
Emivit 50 WP	65.20c	99.21ab	37.18d	73.53b	98.41a	1.98ef
Evavit 80 WG	10.16f	38.26d	7.66e	25.91d	1.98e	7.02e
C Tannin	10.71f	0.00f	1.35e	2.78f	5.83e	2.78ef
Q Tannin	6.07f	0.00f	1.47e	3.85f	0.00e	3.29ef

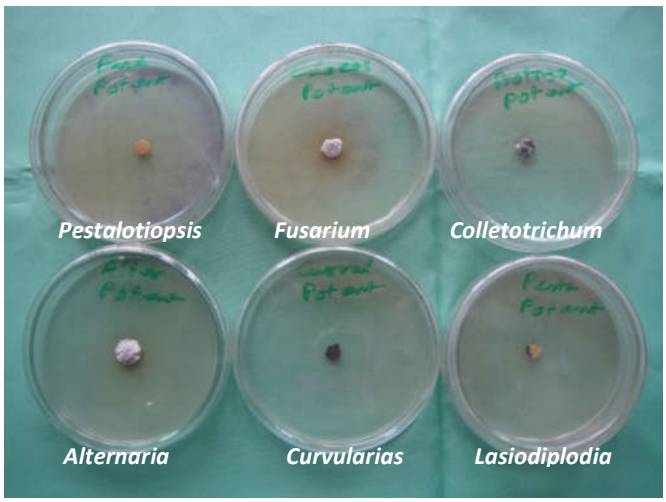
Means within the same column having a common letter (s) do not differ significantly (P=0.05)



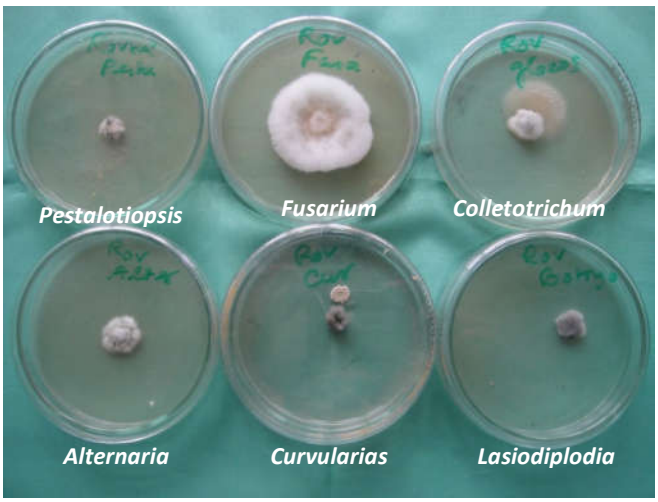
a.Conza



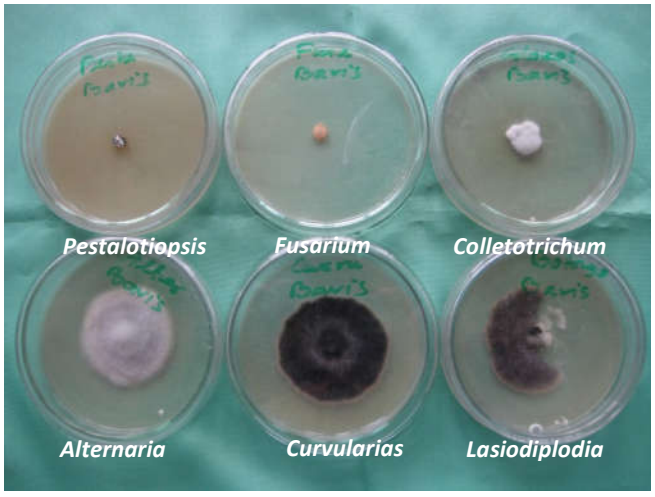
b. Folicur



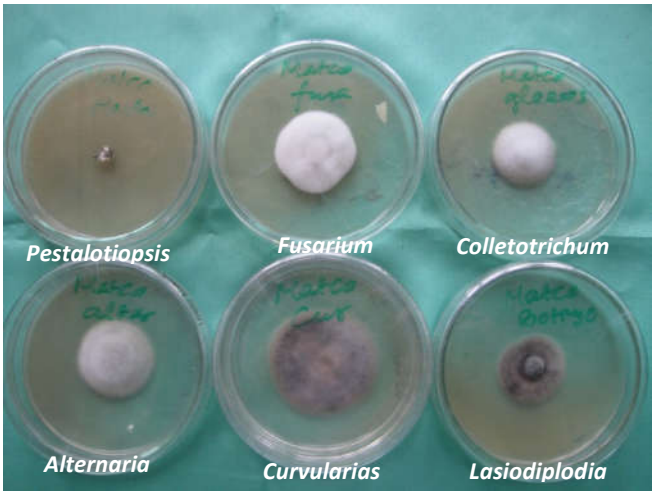
c. Potent



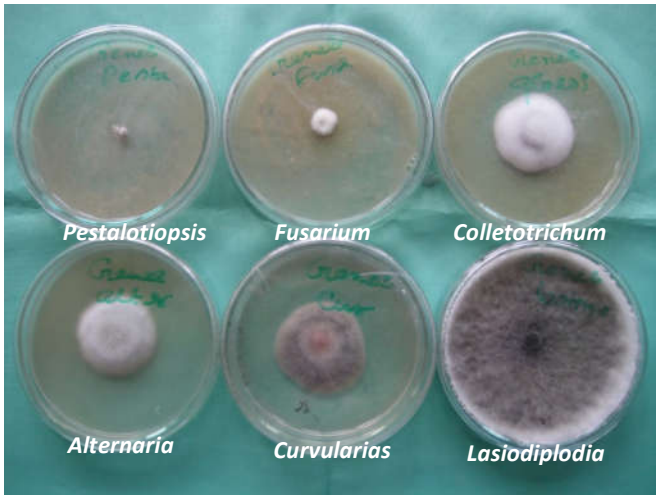
d. Rovral



e. Bavistin

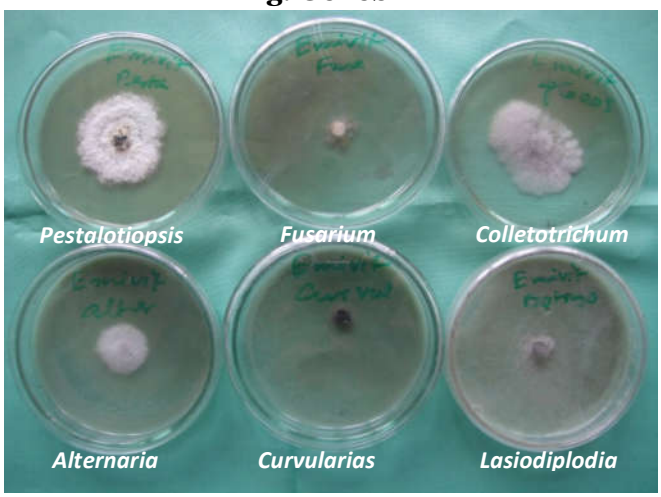


f. Matco



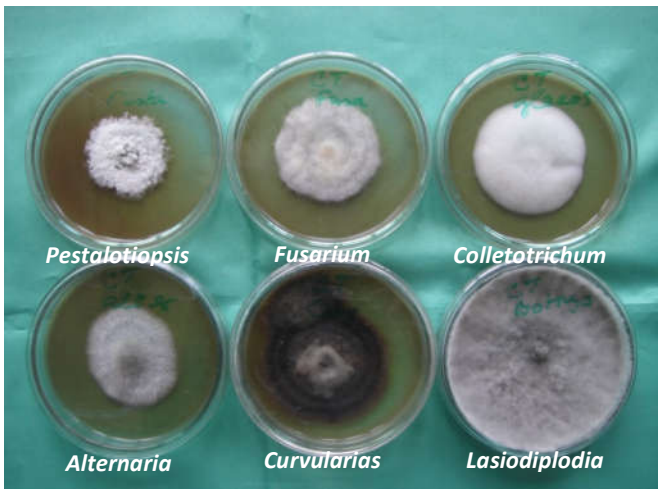
g. Geneb

h. Kasumin



i. Emivit

j. Evavit



k. C Tanin

l. Q Tannin

m. Control

Figure 1. In vitro control of six major fungi causing fruit spot and rot of Indian jujube using different fungicides and two Tannins

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