Agro-Science Journal of Tropical Agriculture, Food, Environment and Extension
Volume 13 Number 1 January 2014 pp. 31 - 36
ISSN 1119-7455

INVITRO INHIBITION OF RADIAL GROWTH, SPORULATION AND GERMINATION OF *ALTERNARIA SOLANI* BY SOME FUNGICIDES

***Ugwuja[1], F. N., Maduewesi[2], J. N. C, Ugwoke,[3,] K. I. and Mbadianya[4], J. I.**
[1]Department of Plant Science and Biotechnology, Michael Okpara University of Agriculture, Umudike.
E-mail: njideka712@yahoo.com
[2]Department of Plant Science and Biotechnology, University of Nigeria, Nsukka.
[3 &4] Department of Crop Science , University of Nigeria, Nsukka.
Corresponding author 's e-mail

ABSTRACT
One laboratory study was carried out at the Department of Plant Science and Biotechnology, University of Nigeria, Nsukka to test the efficacy of these three fungicides; Antracol, Benlate and Captan in the control of Alternaria solani with a view of recommending the potent ones to farmers for use in the control of disease caused by Alternaria species. Cultures of A. solani isolated from diseased tomato plants were used for the study. Effects of the fungicides on radial growth and sporulation were investigated. The cultured plates were set up in a completely randomized design in four replicates. Radial growth measurements were determined by taking the diameter of the colony in each cultured plate along two equatorial axes of the plate and their average recorded for all four replicates of a given concentration to the nearest millimeter. Antracol, Benlate and Captan inhibited radial growth, sporulation and germination of A. solani. Captain was the most effective of the fungicides in inhibiting radial growth causing 46.80, 63.29, 68.94 and 73.75 percent inhibition at 10, 100, 200 and 500 ppm respectively. These inhibitions were significantly greater (p= 0.05) than those of Benlate and Antracol at the same levels followed by Benlate while Antracol was the least. Therefore, the percent intibition in vitro clearly showed that among three fungicides tested, Captan was the most effective and has the best potential of being employed in the control of A. solani in the field or in the green house.

Keywords: Alternaria, control, disease, fungicides and potato

INTRODUCTION
Alternaria solani Sorauer, the casual organism of early blight of potato (*Solanum tuberosum*) and tomato (*Lycopersicon esculentum*) is a common and frequently serious pathogen of these food crops in most parts of the world where these crops are important (Mathur and Shekhawat, 1986; Agrios, 2005; Chaerani and voorrips, 2006 and Majeed *et al*., 2014). Its destructive effect on crops has been recorded from Mexico to Canada in North America, Java, Bermuda, Eastern United States, Pennsylvania and Africa (Keinath *et al.*, 1996; Poya, 1996, Agrios, 2005; Olanya et *al.*, 2009; whiting *et al*., 2013).

It is wide spread in distribution but most important in the warm regions of the world. It is prevalent in cultivated soils and mostly found as saprobe on dead decaying plants and in soil from which the conidia are picked and invade laboratories as contaminants (Agrios, 2005). Infection by *A. solani* is characterized by the appearance of dark or almost black, more or less circular dead areas or spots upon the leaflets, which show a concentric series of rings giving the lesion a target board effect. Adjacent spots subsequently enlarge and coalesce to cover much surface of the leaf forming more extended dead areas and seriously infected plants suffer premature defoliation (Abdalla *et al*., 2014; Wiggins, 2014; Chaerani and Voorrips, 2006; Pandey, 2003). According to these authors, the disease causes large economic loss to farmers especially when the season begins with abundant moisture or frequent rains followed by warm and dry weather which are unfavorable for the host and help in rapid disease development.

Yield losses up to 79% due to early blight disease have been reported from Canada, India, the United States and Nigeria (Chaerani and Voorrips, 2006). The fungus can cause disease on the foliage (leaf blight), stem (collar rot) and fruit, and can result in severe damage

http://dx.doi.org/10.4314/as.v13i1.5

during all stages of plant development. The leaf blight phase, commonly referred to as early blight, is the most important phase of the disease and can result in complete loss of the crop when incidence is severe. *A. solani* has also been implicated in causing early blight of lettuce (*Lactuca sativa* L.), eggplant (*Solanum aethiopicum*) bell pepper and hot pepper (*Capsicum* spp.) and other members of the Solanaceae (Whiting *et al.*, 2013 and Wiggins, 2014).

The genus *Alternaria* contains a number of species that are widely involved in disease of plants, animals and man. For instance *A. solani* produces a highly phytotoxic antibiotic (alternaric acid) which causes wilting in higher plants and also contaminates human food (Rossman *et al*., 1990, Hawksworth *et al*., 1995). They survive on a wide range of substrate and have been isolated from plant sources.

Among plants, diseases caused by these species are leaf spot of cabbage and cauliflower by *A. brassicola*, leaf spot of cucumber and melon by *A. cucumerina* , black rot of carrot by *A. radicina*, leaf blight of carrot by *A. dauci* and rot of citrus fruit by *A. citri* (Laemmlen 2001; Kucharek 2000 and Rossman *et al.*, 1990). In addition, *Alternaria* species have been implicated in a newly discovered disease of sweet potato (*Ipomoea batatas)* known as leaf spot and stem blight of sweet potato caused by *A. bataticola* (Lopes *et al*., 1994).

Control of early blight on crops has been attempted traditionally by use of cultural methods including crop rotation and removal and destruction of infected leaves and stems. While these methods have been helpful to some extent, the level of success achieved has not been adequate to meet the growing demands for these crops. Fungicides treatments are generally the most effective control measures for early blight disease (Chaerani and Voorrips, 2006). Fortunately, some fungicides including Maneb, Zineb, Mancozeb, Chlorothalonil, thiram, Capan, Fludioxonil, Iprodione and Imazalil have been recommended by some authors (Laemmlen, 2001 and Whiting *et al*., 2013) for control of *Alternaria* diseases. Reports of effective use of Antacol, Benlate and Captan to control other fungal diseases are well documented (Hamini-kadar *et al*., 2014; Pscheidt, 2014; Govindappa *et al*., 2011; Zafar *et al*., 2010; Raziq *et al*., 2008; Nawar, 2007; Goertz 2004; Gupa and Aneja 2001; Khokar and Jaffery, 2000; Amadioha, 1998 and Casa *et al*., 1998).

Although the performance of several fungicides against *Alternaria* diseases has been determined *in vitro* and *in vivo* by many researches, there are scanty information on the effects of Antracol, Benlate and Captan on radial growth, sporulation and germination of *Alternaria solani*. Therefore, studies were carried out in the laboratory at the University of Nigeria, Nsukka, Nigeria to test the efficacy of these fungicides in the control of *A. solani* with a view to recommending the potent ones to farmers for use in the control of disease caused by *Alternaria* species.

MATERIALS AND METHODS

Cultures of *A. solani* previously isolated from diseased tomato plants and maintained on Potato Dextrose Agar (PDA) slants in the Department of Botany, University of Nigeria, Nsukka, Nigeria, were used in this experiment. The fungicides Antracol (Zinc propylene bis-dithio-carbamate), Benlate (Methyl-1-butlyl (carbamoy1) 2- benzimidazole carbamate), and Captan (N-trichloromethyl thio)-4- cyclohexane 1, 2-dicarboximide) were also obtained from the same place and used in the experiment.

The effects of the fungicides on radial growth and sporulation were investigated on Potato-Dextrose Agar (PDA). The PDA was before use autoclaved for 20 minutes at pressure of 1.1kg/cm^2) and temperature (121^{0}c). Each fungicide was weighed, suspended in sterile distilled water and added to molten PDA in flask at approximately 50^{0}c in proper amounts to achieve final concentration of 10, 100, 200 and 500 ppm (parts per million active ingredients). The media to which no fungicides were added served as the controls. The flasks containing the different fungicides levels and autoclaved media were thoroughly shaken to ensure uniform distribution of the fungicides and 20ml each of the fungicide level was dispensed into each four 90mm Petri dishes. The media after solidifying were inoculated each with a 2mm mycelial disc cut from the periphery of a 7-day old culture of *A. solani* as described by Ugwuja and Chiejina (2011). The culture plates were set up in completely randomized design in four replicates and incubated for seven days at room temperature (27±4^{0}C). Radial growth measurements were done by taking the diameter of the colony in each culture plate along two equatorial axes of the plate and their average recorded for all four replicates of a given concentration to the nearest millimetre.

The same plates from which radial growth measurements were taken were used for the sporulation test. After eight days of incubation, each plate was flooded with 8ml of distilled water to form conidia suspension. The conidia were dislodged using sterile camel's hair brush and filtered through a double layer of cheese cloth to remove mycelia fragments. The spore concentration in both the control plates and fungicide amended plates were assessed with the aid of the Neubauer's improved haemacytometer to obtain actual spore amounts and to determine the extent to which each fungicide inhibited sporulation.

The effect of the fungicides on spore germination of *A. solani* was assessed on Potato-Dextrose Broth (PDB). Five millilitre of conidia suspension obtained earlier was centrifuged, the supernatant poured away and the

residues (spores) used immediately for germination test. Four levels (10, 100, 200, and 500ppm) of each fungicide were made, each level was dissolved in appropriate amount of PDB and 0.05ml each of a fungicide level was placed in each four microscope slide in a plate lined with moistened filter paper. Using the tip of a transfer needle, some 500 conidia were added to each medium on a slide from the spore residues and thoroughly mixed. The mixtures were incubated for two hours at room temperature (27 ± 4^0C). At the end of this period, germinated spores were counted on each slide by means of a hand operated tally counter. Spores were considered germinated if the germ tube was as long as the spore or exceeds half the diameter of the spore as described by Nene *et al*. (1979) and Munkvold (1993). On each slide 200 spores were counted and the percentage of those that had germinated determined. All relevant data were subjected to analysis of variance (ANOVA) and mean separation done using Fischer's least significant difference (F-LSD).

RESULTS AND DISCUSSION

Antracol, Benlate and Captan inhibited radial growth, sporulation and germination of *Alternaria solani*. The effects of the various levels of the fungicides on radial growth of the fungus on PDA after seven days of inoculation are shown in Table (1). The analysis of variance showed significant differences in the treatment means. Percentage inhibition increased as the colony diameter decreased and as the concentrations of the fungicides increased. The colony diameter, a measure of the radial growth was inhibited to various extents by all concentrations of the fungicide tested. Captan was the most effective of the fungicides in inhibiting radial growth causing 46.80, 63.29, 68.94 and 73.75 percent inhibition at 10, 100, 200 and 500ppm respectively. These inhibitions were significantly greater (P=0.05) than those of Benlate and Antacol at the same levels. Benlate was the next in efficacy and caused 20.38, 27.12, 29.43 and 51.77 percent inhibition of radial growth at the same levels while Antracol was the least (Table 1).

The effects of the fungicides on sporulation (conidial production) of the fungus on PDA after eight days of incubation are shown in Table (2). Sporulation was similarly inhibited to various extents by all the levels of the fungicides. Captan again was the best of the fungicides in this regard followed by Benlate and Antracol. Although Captan was most effective than Benlate and Antracol, in this regard, the spore concentration under these treatments differed slightly from each other as shown in Table (2).

Table (3) shows the effect of the fungicides on spore germination of *A. solani* on PDB after 2 hours of incubation at room temperature. The percentage germinations and percentage inhibitions revealed that Captan was the most effective of the fungicides causing only two percent germination at 10ppm and no germination at other levels. Benlate was the next in efficacy to Captan followed by Antracol. The results showed that at a lower concentration of 100ppm, Captan gave maximum inhibition of germination. In the overall investigations, the best interaction was achieved by Captan at 500pmm.

The use of protectant or eradicant fungicides to control fungal diseases depends on their ability to inhibit germination, growth and sporulation (Mehrotra and Aggarwal, 2003). From the present study Captan exhibited the greatest fungitoxic effect on *A. solani*. This result corroborates the findings of Ellis *et al.* (2011); Govindappa *et al*., (2011); Sugha *et al*. (1995) working separately on different *Fusarium* species. However, Zafar *et al.* (2010) in their studies on mango malformation caused by *F. mangiferae* found that Captan exhibited minimal effect on colonies of *F.mangiferae* *in vitro* test whereas Benlate gave excellent control. The reports of Aveling and Snyman (1993); Biggs *et al*, (1993) were also at variance with the result of the present study. Filajdic and Sutton (1992) reported that Captan could not control *Alternaria* blotch of apples caused by *A. mali*.

Table 1: Effects of Antracol Benlate and Captan on Radial growth of *A. solani*

Fungicides levels (ppm)	Antracol		Benlate		Captan	
	Colony diameter (mm)*	Percentage inhibition	Colony diameter (mm)*	Percentage inhibition	Colony diameter (mm)*	Percentage inhibition
0	70.50	0.00	70.50	0.00	70.50	0.00
10	59.50	15.60	56.13	20.38	37.50	46.80
100	53.13	24.1	51.38	27.12	25.88	63.29
200	51.25	27.30	49.75	29.43	21.88	68.94
500	36.88	47.69	34.00	51.77	18.50	73.75
LSDS(P=0.05)	3.89		3.71		2.29	

*The values are means of four replicates determined 7days after inoculation

Table 2: Effects of Antracol Benlate and Captan on sporulation of *A. solani*

	Antracol		Benlate		Captan	
Fungicide levels (ppm)	Spore conc./ml*	Percentage inhibition	Spore conc./ml*	Percentage inhibition	Spore conc./ml*	Percentage inhibition
0	92.88	0.00	92.88	0.00	92.88	0.00
10	78.25	15.75	60.63	34.72	49.00	47.24
100	63.38	31.76	49.75	46.43	34.75	62.58
200	50.08	46.08	40.13	56.79	25.00	73.08
500	41.25	55.58	33.56	63.86	16.63	82.09
LSDS(P=0.05)	30.03		25.13		24.92	

* The values are means of four replicates determined 8 days after inoculation.

Table 3: Effects of Antracol, Benlate and Captan on germination of *A. solani*

	Antracol		Benlate		Captan	
Fungicides levels (ppm)	Percentage germinat-ion	(%) inhibition	Percentage germination	(%) inhibition	Percentage Germination	(%) inhibition
0	100.00	0.00	100.00	0.00	100.00	0.00
10	64.66	35.24	54.33	45.67	2.00	98.00
100	29.66	70.34	23.66	76.34	0.00	100.00
200	26.16	73.84	14.66	85.34	0.00	100.00
500	7.66	92.32	4.50	95.50	0.00	100.00
LSDS(P=0.05)	5.61		3.41		0.42	

*****The values are means of four replicates determined 2 hours after inoculation.

at variance with that of Raziq *et al*. (2008), Khokhar and Jaffrey (2000) and Mohammad *et al*. (1990) who reported that Antracol was highly effective against *Peronospora* destructor.

The observed differences in the performance of these fungicides could be due to detoxification before the site of action has been reached, lack of conversion of a compound into the fungi-toxic principal or the production of inhibitory factor (Cremlyn, 1991). Moreover, various species of fungi show great variation in their ability to resist or tolerate the actions of fungicide in different environments (Agrios, 2005).

Although Benlate achieve limited inhibition of growth, sporulation and germination of *A. solani*, it has been reported to be a highly effective and broad spectrum systemic fungicide against *Fusarium solani*, *and Rhizoctonia solani (*Nawar, 2007).

In our study, radial growth, sporulation and germination of *A. solani* were inhibited at higher concentration of Antracol. Similar results were observed by Hamini-Kadar *et al*. (2014), Pathan *et al*., (2005) and Rajput *et al*. (2012). However, this result is

In view of this, there is need for further studies on these fungicides in the field or green house in order to ascertain their full potential in the control of *A. solani*.

We observed that germination of *Alternaria* spores was totally inhibited by Captan at a lower concentration of 100ppm. This suggests that Captan can effectively serve as soil drench to control germination of *Alternaria* spores at that level of active ingredient. The results also indicate that at higher levels (up to 500ppm) Captan can effectively control already developed disease and stop its spread in the field.

In conclusion, therefore, the percent inhibition *in vitro* clearly showed that among the three fungicides tested Captan was the most effective and has the best potential of being employed in the control of *A. solani* in the field or in the green house.

REFERENCES

Abdalla, S. A., Algam, S. A. A., Ibrahim, E. A. and Naim, A. M. E. (2014). *In vitro* screening of *bacillus* isolates for biological control of early blight disease of tomato in Shambat soil. *World Journal of Agricultural Research*, *2*(2), 47-50.

Aggarwal, A. and Mehrotra, R. S. (2003). *Plant Pathology*. (2nd edition). Tata McGRAW – Hill Publishing Company Limited, New Delhi.846pp.

Agrios G. N. (2005). *Plant Pathology*. (5th edition). Academic Press. 922pp.

Amadioha A. C. (1998). Control of postharvest tuber rot of potato incited by *Rhizoctonia bataticola*. *Archieve of Phytopathology and plant protection* 31:225-231.

Aveling, T.A. and Snyman,H.G. (1993). Evaluation of seed treatment for reducing *Alternaria porri* and *Stemphylium vesicularium* on onion seed. *Plant Diseases* 79: 1009-101.

Biggs, A.R., Solihati, W.D. and Ingle, I. (1993). Control of *Alternaira* infection of ruti of apples cultivar Nittany with calcium chloride and fungicides. *Plant Disease* 77: 976-980.

Casa, R. T., Zambolin, L. and Reis, E. M. (1998). Transmission and control of *Diplodia* in maize seed. *Brazilian Phytopathology* 23:225-231.

Chaerani, R. and Voorrips, R.E. (2006).Tomato early blight (*Alternaria solani*): the pathogen, genetics and breeding for resistance. *Journal of General Plant Pathology* 72: 335-347

Cremlyn, R. J. (1991). *Agrochemicals:* preparation and mode of action. John Wiley and Sons, Chichester, New York. 296pp.

Ellis M. L., Broder, K. D., Paul, P. A. and Dorrance A. E. (2011).Infection of soybean seed by *Fusarium gramminearum* and effect of seed treatments on disease under controlled condition. *Plant disease* 95:401-407.

Filajdlic, N. and Sutton, T. B. (1992). Chemical control of Alternaria blotch of apples caused by *Alternaria mali Plant Disease* 76 126-130.

Goertz, A., Yang, X., Vaupel, M. Oshima, A. and Dias, L. (2004). Antracol® – A fungicide improving zinc nutrition in plants. Available on *andreas.goertz@bayer.com*

Gupta, A. and Aneja, K. R. (2001). Field efficacy of seed dressing chemicals on seedling emergence, seed yield and seed weight in soybean. *Indian Phytopathology* 28(1): 54-58.

Govindappa, M. Ravishankar, V. R. and Lokesh, S. (2011). *In vitro* and *In vivo* responses of different treating agents against wilt disease of safflower. *Journal of Cereals and Oilseeds* Vol. 2(1) 16-25.

Hamini-kadar, N. Benouli, H., Benichou, S., Kihal, M., Benali, S. and Henni, J.E. (2014). *In vitro* effects of two fungicides on pathogenic fungi causing root rot of tomato in Algeria. *Journal of Agricultural Research* 9:2584-2587.

Hawksworth, D.L. kirik, P.M., Sutton, B.C. and Pegler, D.N. eds (1995). *Anisworth* and *Bisby's dictionary of the fungi.* CAB international, wallinford, oxford. 616pp.

Khokhar, L. k. and Jaffrey, A. H. (2000).Efficacy of fungicides against downy mildew and yield of onion. *Pakistan Journal of Agricultural research* 16:1

Keinath, A. P., DuBose, V. B. and Rathwell, P. J.(1996). Efficacy and economics of three fungicides application schedules for early blight control and yield of fresh market tomato. *Plant Disease* 80:1277-1282.

Kucharek, T. (2000). *Alternaria* diseases of Crucifers. Florida cooperative extension service, Institute of Food and Agricultural Sciences, University of Florida.

Laemmlen, F. (2001). *Alternaria* Diseases.University of California Cooperative Extension. Available on, *http://anrcatalog ucdavus.edu*

Lopes, C.A., and Boteux, L.S. (1994). Leaf spot and stem blight of sweet potato caused by *Alternaria bataticola, plant Disease:*78 1107-1109.

Majeed, A., Ahmad, H. and Khan, H. (2014). Effect of systemic and contact fungicides on late blight disease and tuber yield of potato. *Journal of Agricultural Technology* 10(1):209-217.

Mathur, K. and Shekhawat, K. S. (1986). Chemical control of early blight in kharif sown tomato. *Indian Journal of Mycolology and Plant Pathology*, 16 (2), 235-236.

Mohammad, T., Muhibullah., M. S. and Saijullah (1990). The effect of different spray fungicides on downy mildew and yield of onion. *Sarhad Journal of Agriculture* 6*:* 377-380.

Nawar, L.S. (2007). Pathological and rhizospherical studies on root-rot disease of squash in Saudi Arabia and its control**.** *African Journal of Biotechnology* **6** (3): 219-226.

Nene, V.I. and Thapliyal, P.N. eds. (1979). *Fungicide in plant disease control.* Oxford and IBH publishing company. New Delhi 507pp.

Sugha, S. K. (2000). Management of white rot of pea by fungicides. *Journal of Mycolology and Plant Pathology* **31**(3): 335-338.

Sugha, S .K. Kapoor, S. K., Singh, B. M. (1995). Management of chickpea wilts with fungicides. *Indian Phytopathology* 48(1): 27-35.

Olanya, O. M. and others (2009). The effects of cropping systems and irrigation management on development of potato early blight. *Journal of General Plant Pathology* 75:267-275.

Pandy, k. k. and others (2003).Resistance to early blight of tomato with respect to various parameters of disease epidemics. *Journal of General Plant Pathology* 69: 364-371.

Pathan M. A.,Leghari T. N., Jiskani, M. M. and Wagan, k. H. (2005). Evaluation of different fungicides against Botyrodiplodia theobromae causing mango gummosis. *International Symposium on Plant Disease Management*, December 20-22, Karachi, P.68

Poysa, V. (1996). Response of cultivar and breeding line of lycopersicon spp to *Alternaria solani*. *Canadian plant disease survey* 76: 5-8.

Rajput, N. A.,Pathan M. A.,Lodhi, A. M., Dou, D. Liu, T., Rain, M. S. and Rajar, F. U. (2012). *In vitro* evaluation of various fungicides against *Fusarium solani* isolated Dalbergia sisso die back. *African Journal of Microbiological Research* 6(27):5691-5699.

Raziq, F. Alam, I. Ishrat, N. and Hakim, K. (2008).Evaluation of fungicides for

controlling downy mildew of onion under field condition. *Sarhad Journal of Agriculture* 24:1

Rossman, A. Y., Palm, M. E. and Spielman, L. J. eds. (1990). *A literature guide for the identification of plant pathogenic fungi.* APS press. St. Paul, Minnesota. 252pp.

Ugwuja, F. N. and Chiejina, N. V. (2011). Preliminary investigation of the causes of leaf rot of cocoyam in Nsukka zone of Nigeria. *Nigerian Journal of Plant Protection* 25:203-213.

Whiting, D., O'Meara, C. and Wilson, C. (2013).Tomato early blight. Colorado Master Gardener Program, Colorado State University Extension. Available on *www.cmg.colostate.edu*

Zafar, I., Mohammad, Y. and Ahmad, S. (2010).Determination of Minimum inhibitory concentration of fungicides against *Fusarium mangiferae*.*Pakistan Journal of Botany* 42(5):3522-3532.