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In vitro Inhibition of Conidial Germination of *Colletotrichum gloeosporioides* Penz. by Fungicides, Plant Extracts and Phytohoromones

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Abstract: Six fungicides viz., cupravit, thiovit, dithane M-45, bavistin, rovral and suncozeb were tested against *C. gloeosporioides*. Dithane M-45, rovral, thiovit and suncozeb were the most effective ones. Cupravit and bavistin were less effective in the inhibition of conidial germination. Ten plant-extracts considered as fungicides were tested, out of which *Tagetes erecta* leaf and *Azadirachta indica* bark extracts were the most effective in inhibiting *Colletotrichum gloeosporioides* after 5-30 minutes of immersion and in 5:1.25 (w/v) concentration. The phytohormone IAA had great effect against the inhibition (100%) of *C. gloeosporioides* at 0.005 to 0.006% concentration for an immersion after 5-30 minutes. Rest of the hormones also had good effects against *C. gloeosporioides*.

Key words: Mango, fungicides, plant extracts, phytohormones, *Colletotrichum gloeosporioides*

Introduction

Anthracnose, also known as blossom blight or leaf spot or fruit rot, is a common, destructive and wide spread diseases in all mango growing countries (Prakash and Srivastava, 1987). Various manifestations of the disease on mango include blossom blight, peduncle blight, leaf spot, twig blight, wither tip, fruit russeting or staining and fruit rot (Singh, 1983). Use of fungicides is common in disease management. Extracts of some plant parts also exhibited marked antifungal effect on germination of fungal spores (Singh and Singh, 1981; Singh *et al.*, 1983; Dubey, 1991 and Alam *et al.*, 1999). Effects of some plant growth regulators were also tested on fungal growth (Sheshtawi and Kiss, 1975). The present experiment was carried out for *in vitro* control of the causal agent (*Colletotrichum gloeosporioides* Penz.) of mango anthracnose by fungicides, plant extracts and phytohormones to reduce the germination and growth.

Materials and Methods

The pathogen *Colletotrichum gloeosporioides* was isolated from Langra variety of mango (*Mangifera indica* L.) and cultured on PDA. Conidia were taken from 10 days old culture on PDA and conidial suspension was made separately in different concentrations (0.05, 0.10, 0.15, 0.20 and 0.25%) of selected fungicides such as, thiovit (sulphur fungicides), cupravit (copper oxychloride), dithane M-45 (manganous ethylene bisdithiocarbamate + zinc sulfate), bavistin (methyl-2-benzimidazole carbamate), rovral (eprodion) and suncozeb. The suspension of fungicides with conidia was taken in sterilized watch glass and kept at 30 ± 2°C for 5-30 minutes. A drop of fungicide treated conidial suspension was taken on separate slides, continue 5 minutes interval and kept in moisture chamber at 30 ± 2°C for 24 hours of incubation. Then a drop of lactophenol cotton blue was placed on conidial suspension on the slides. The slides were examined under high power (×40) for recording the percentage of conidial germination.

Extraction of root, seed, bark and leaf tissue in alcohol was done following the method of Mahadevan and Sridhar (1982). Five gram tissues were cut into pieces and immediately plunged in boiling ethyl alcohol (80%) in a beaker and allowed to boil for 5-10 minutes using five to ten ml of alcohol for each gram of tissue. The extracts were done on top of a steam bath. The extracts were cooled in a pan of cold water. The tissues were crushed thoroughly in a mortar with a pestle and then passed through two layers of cheese-cloth. Re-extracted ground tissues for 3 minutes in hot 80% alcohol and 2-3 ml of alcohol were used for every gm of tissues. The extracts were cooled and passed through cheese-cloth and filtered through Whatman's No. 1 filter paper. The volume (10 ml) of the extracts were evaporated on a steam bath to dryness and 1.25 ml of sterilized distilled water was added for five grams of tissues and the extracts were used as fungicides.

Conidia from the culture on PDA plates were taken and conidial suspensions were made separately with different plant extracts (*Datura metel* leaf and seed, *Tagetes erecta* leaf and root, *Vinca rosea* leaf, *Azadirachta indica* leaf and bark, *Allium sativum* bulb, *Leonurus sibiricus* leaf and *Cassia alata* leaf). These suspensions (1.25 ml) were taken in small sterilized petri dishes (65 mm) and were kept at 30 ± 2°C for 5-30 minutes. A drop of treated conidial suspension (from different plant extracts) was taken on separate slides continue 5 minutes interval and were kept at 30 ± 2°C in a moisture chamber for 24 hours of incubation. Then a drop of lactophenol cotton blue was placed on the conidial suspension on slides. The slides were examined under high power (×40) for recording the percentage of conidial germination of *C. gloeosporioides*.

Conidia were taken from 10 days old culture on PDA and conidial suspension was made separately in different concentrations (0.001, 0.002, 0.003, 0.004, 0.005 and 0.006%) of selected phytohormones namely, NAA (α- Naphthyl acetic acid), IAA (Indol acetic acid) and 2,4-D (2, 4-Dichloro phenoxy acetic acid). These suspensions were taken in sterilized petri dishes (65 mm) and kept at 30 ± 2°C for 5-30 minutes. A drop of treated conidial suspension from different concentrations of phytohormones was taken on separate slides continue 5 minutes interval and were kept in a moisture chamber at 30 ± 2°C for 24 hours of incubation. Then a drop of lactophenol cotton blue was placed on conidial suspension on each slide. The slides were examined under high power (×40) for recording the percent germination. Statistical analysis of data given as percentage was carried out from angular transformed values and performed using Microsoft Excel software. LSD were determined, whenever, the calculated 'F' values were significant at 5% level (Snedecor and Cochran, 1980).

Results and Discussion

Out of six fungicides tested, dithane M-45, rovral and suncozeb were the most effective against *Colletotrichum gloeosporioides*, when the fungus was immersed for 5-30 minutes at 0.05-0.25% concentrations. Hundred percent conidial germination inhibition occurred after treating the three (dithane M-45, rovral and suncozeb) fungicides in all cases of immersion period and concentrations. Rest of the three fungicides (thiovit, cupravit and bavistin) have good inhibitory effect (66, 94 and 91%) against *C. gloeosporioides* at the concentration of 0.05% after an immersion duration of 30 minutes. The germination was completely checked (100%) after 25 minutes of application of 0.25% cupravit. The 0.20% concentration has fairly good effect on the inhibition of this fungus with the application of these three fungicides (thiovit, cupravit and bavistin Table 1). Hundred per cent germination inhibition occurred with the application of thiovit and bavistin after 15 minutes of immersion and 25 minutes in cupravit at 0.25% concentration. With the increase in concentration and immersion

Table 1: Relation of concentrations and exposure time to effectiveness of various fungicides against *Colletotrichum gloeosporioides*.

Name of fungicides	Concentrations (%)	Percentage of conidial germination[1].						Correlation (r1)	Calculated F value (5%)		LSD (0.05)
		5	10	15	20	25	30		Concentration	Immersion period	
Thiovit	0.05	96(4)	85(15)	70(30)	62(38)	45(55)	34(66)	0.994	535.95 *	82.25*	6.842
	0.10	82(18)	54(46)	43(57)	37(63)	25(75)	18(82)	0.963			
	0.15	65(35)	48(52)	40(60)	28(72)	17(83)	10(90)	0.601			
	0.20	5(95)	3(97)	2(98)	1(99)	0(100)	0(100)	0.978			
	0.25	4(96)	2(98)	0(100)	0(100)	0(100)	0(100)	0.845			
Cupravit	0.05	58(42)	35(65)	20(80)	12(88)	8(92)	6(94)	0.958	46.57*	37.45*	7.156
	0.10	16(84)	12(88)	15(85)	9(91)	6(94)	5(95)	0.927			
	0.15	15(85)	10(90)	10(90)	8(92)	5(95)	4(96)	0.975			
	0.20	12(88)	9(91)	6(94)	5(95)	3(97)	2(98)	0.994			
	0.25	10(90)	7(93)	3(97)	2(98)	0(100)	0(100)	0.979			
DithaneM-45	0.05	0(100)	0(100)	0(100)	0(100)	0(100)	0(100)	0	-	-	-
	0.10	0(100)	0(100)	0(100)	0(100)	0(100)	0(100)	0			
	0.15	0(100)	0(100)	0(100)	0(100)	0(100)	0(100)	0			
	0.20	0(100)	0(100)	0(100)	0(100)	0(100)	0(100)	0			
	0.25	0(100)	0(100)	0(100)	0(100)	0(100)	0(100)	0			
Bavistin	0.05	93(7)	35(65)	26(74)	19(81)	14(86)	9(91)	0.859	219.14*	166.88*	4.748
	0.10	38(62)	25(75)	16(84)	14(86)	12(88)	7(93)	0.961			
	0.15	24(76)	20(80)	12(88)	13(87)	10(90)	5(95)	0.963			
	0.20	19(81)	15(85)	8(92)	10(90)	9(91)	3(97)	0.909			
	0.25	13(87)	10(90)	0(100)	0(100)	0(100)	0(100)	0.840			
Rovral	0.05	0(100)	0(100)	0(100)	0(100)	0(100)	0(100)	0	-	-	-
	0.10	0(100)	0(100)	0(100)	0(100)	0(100)	0(100)	0			
	0.15	0(100)	0(100)	0(100)	0(100)	0(100)	0(100)	0			
	0.20	0(100)	0(100)	0(100)	0(100)	0(100)	0(100)	0			
	0.25	0(100)	0(100)	0(100)	0(100)	0(100)	0(100)	0			
Suncozeb	0.05	0(100)	0(100)	0(100)	0(100)	0(100)	0(100)	0	-	-	-
	0.10	0(100)	0(100)	0(100)	0(100)	0(100)	0(100)	0			
	0.15	0(100)	0(100)	0(100)	0(100)	0(100)	0(100)	0			
	0.20	0(100)	0(100)	0(100)	0(100)	0(100)	0(100)	0			
	0.25	0(100)	0(100)	0(100)	0(100)	0(100)	0(100)	0			

r2 - 0.965, 0.881 and 0.970 for thiovit, cupravit and bavistin respectively. In other cases correlation was not present.
[1] - Mean of three replications. r1 - Correlation between immersion period and germination inhibition of conidia.
r2 - Correlation between concentrations and germination inhibition of conidia. Parenthesis show percent inhibitions.

Table 2: Effect of different plant extracts as fungicides on conidial germination of *Colletotrichum gloeosporioides* Penz. after immersed for 5 - 30 minutes.

Name of plant extracts	Percentage of conidial germination[1].						Correlation (r3)	Calculated F value (5%)		LSD (0.05)
	5	10	15	20	25	30		Plant extracts	Immersion period	
Datura metel Seed	92(8)	80(20)	73(27)	70(30)	61(39)	50(50)	0.9803	2113.93*	156.52*	4.1243
Datura metel Leaf	95(5)	90(10)	82(18)	80(20)	78(22)	75(25)	0.9483			
Tagetes erecta Root	95(5)	92(8)	85(15)	70(30)	61(39)	57(43)	0.9848			
Tagetes erecta Leaf	0(100)	0(100)	0(100)	0(100)	0(100)	0(100)	0			
Vinca rosea Leaf	97(3)	93(7)	88(12	85(15)	80(20)	75(25)	0.9932			
Azadirachta indica Leaf	90(10)	88(12)	75(25)	70(30)	64(36)	65(35)	0.9482			
Azadirachta indica Bark	0(100)	0(100)	0(100)	0(100)	0(100)	0(100)	0			
Allium sativum Bulb	98(2)	95(5)	93(7)	90(10)	87(13)	85(15)	0.9893			
Leonurus sibiricus Leaf	85(15)	83(17)	80(20)	75(25)	70(30)	68(32)	0.9912			
Cassia alata Leaf	90(10)	86(14)	83(17)	78(22)	76(24)	74(26)	0.9846			

[1] - Mean of three replications. r3 - Correlation between immersion period and germination inhibition of conidia.
Parenthesis show percentage of inhibitions.

period, the inhibition of conidial germination of this fungus also increased (Table 1). Correlation (r1) value (0.601-0.994) indicates that there was highly significant relationship between immersion period and percentage of conidial germination inhibition, except in dithane M-45, rovral and suncozeb. Correlation (r2) value (0.881-0.970) indicates that there was a highly significant relationship between concentrations and conidial germination inhibition, except in dithane M-45, rovral and suncozeb. Calculated F value is greater than table value in all the cases of thiovit, cupravit and bavistin. It indicates that there was a significant difference among the concentrations of each fungicide and immersion periods of conidia in all cases. But in case of dithane M-45, rovral and suncozeb the results are same in all immersion periods and concentrations (Table 1). Hossain *et al.* (2001) reported the efficacy of different fungicides in controlling the purple blotch of onion seed-crop and observed that combined application of rovral 50wp @ 0.2% + redomil MZ-72 @ 0.2 % gave the best control of purple blotch and maximum seed yield of onion followed by individual application of rovral 50wp @ 0.2 % and score 250EC @ 0.05 % when sprayed at an interval of 15 days. Alam *et al.* (2000) reported the effect of fungicides on the inhibition of *Bipolaris sorokiniana* and found bavistin, dithane M-45 and tilt to be the most effective fungicides. They stated that concentrations 500 to 2500ppm and 1/10 to 1/1000 ml were the most effective after 5 to 30 minutes immersion. Alam *et al.* (1999) reported the growth inhibition (*in vitro*) of chilli fruit rot pathogen *Alternaria tenuis* and found redomil, dithane M-45, cupravit, bavistin and rovral to be the most effective against *A. tenuis* when immersed for 5 to 30 minutes at 500 to 2500ppm concentrations. Ahmed *et al.* (1991) evaluated eight fungicides and observed dithane M-45

Table 3: Effect of different phytohormones as fungicides on the inhibition of conidial germination of *Colletotrichum gloeosporioides* after immersed 5-30 minutes

Name of fungicides	Concentrations (%)	Percentage of conidial germination[1].						Correlation (r1)	Calculated F value (5%)		LSD (0.05)
		5	10	15	20	25	30		Concentration	Immersion period	
NAA	0.001	42(58)	35(65)	16(84)	7(93)	4(96)	3(97)	0.968	113.747*	115.988*	5.879
	0.002	40(60)	33(67)	14(86)	6(94)	3(97)	2(98)	0.980			
	0.003	29(71)	24(76)	8(92)	5(95)	2(98)	0(100)	0.985			
	0.004	22(78)	7(93)	5(95)	2(98)	1(99)	0(100)	0.975			
	0.005	6(94)	3(97)	0(100)	0(100)	0(100)	0(100)	0.845			
	0.006	0(100)	0(100)	0(100)	0(100)	0(100)	0(100)	0			
IAA	0.001	35(65)	25(75)	19(81)	10 (90)	7(93)	2(98)	0.996	198.49*	73.51*	4.852
	0.002	32(68)	18(82)	13(87)	9(91)	4(96)	0(100)	0.981			
	0.003	20(80)	14(86)	11(89)	8(92)	7(93)	0(100)	0.915			
	0.004	10(90)	6(94)	0(100)	0(100)	0(100)	0(100)	0.828			
	0.005	0(100)	0(100)	0(100)	0(100)	0(100)	0(100)	0			
	0.006	0(100)	0(100)	0(100)	0(100)	0(100)	0(100)	0			
2,4-D	0.001	45(55)	34(66)	28(72)	23(77)	18(82)	13(87)	0.993	224.966*	71.755*	5.124
	0.002	36(64)	26(74)	22(78)	19(81)	15(85)	9(91)	0.808			
	0.003	28(72)	21(79)	17(83)	13(87)	8(92)	6(94)	0.996			
	0.004	20(80)	16(84)	12(88)	7(93)	5(95)	2(98)	0.996			
	0.005	17(83)	11(89)	9(91)	5(95)	0(100)	0(100)	0.962			
	0.006	0(100)	0(100)	0(100)	0(100)	0(100)	0(100)	0			

r5 - 0.985, 0.970 and 0.972 for NAA, IAA and 2,4-D respectively. [1] - Mean of three replications.
r4 - Correlation between immersion period and inhibition of conidial germination.
r5 - Correlation between concentration of NAA, IAA and 2,4-D and inhibition of conidial germination.
( ) Parenthesis show percent inhibitions.

(suncozeb) to give the best control of anthracnose (*Colletotrichum gloeosporioides* Penz.), followed by Bordeauxe mixture. Teo and Kuch (1982) reported that in laboratory tests, several fungicides were promising against mycelial growth of *Colletotrichum gloeosporioides*, the causal organism of anthracnose. The results of present studies are in accord with the earlier works. Cochrane (1958) reported that there was no useful universal fungicide. A fungicide, which is lethal or highly toxic to a particular fungus, may be totally ineffective against another fungus even at higher concentrations. Present findings show that thiovit, bavistin and cupravit were less effective and dithane M-45, rovral and suncozeb were highly effective against *C. gloeosporioides*. The results of the present investigation are in the same opinion with the comments of Cochrane (1958) and similar result was reported by Sekhare *et al.* (1989).

Ten plant extracts were tested as fungicides. Hundred percent conidial germination was inhibited for immersion after 5-30 minutes at 5:1.25 (w/v) concentration in *Tagetes erecta* leaf and *Azadirachta indica* bark extracts after 24 hours of inoculation. In *Dutura metel* seed extracts, 50% conidial germination inhibited after 30 minutes of immersion. Rest of the plant extracts have intermediary effect against *C. gloeosporioides*. Among the plant extracts, *Alium sativum* extract was less effective (15% inhibition after 30 minutes) on the germination of this fungus. Present study shows that there is an inactivation effect of plant extracts against *Colletotrichum gloeosporioides*. Correlation (r3) value (0.982-0.9932) indicates that there was highly significant relationship between immersion period and conidial germination inhibition, except *Tagetes erecta* leaf and *Azadirachta indica* bark extracts. Calculated F value is greater than table value in case of immersion period and different types of plant extracts. It indicates that there was a significant difference among plant extracts and conidial germination inhibition of all immersion periods (Table 2). Alam *et al.* (1999) reported the antifungal effects of leaf and root extracts of *Vinca rosea* and leaf, root and seed extracts of *A. indica* against chilli fruit rot pathogen *A. tenuis*. Chauhan and Joshi (1990) reported the efficacy and persistence of 14 plant extracts and found that carbendazim (0.05) as mango fruit dip treatments in controlling the mango fruit anthracnose (caused by *Colletotrichum gloeosporioides*) was the most effective control treatment. Eucalyptus oil (2%) and castor oil (10%) solutions inhibited infection for > 2 weeks when fruit were inoculated and were significantly better than other plant extracts tested. Castor oil (5%), eucalyptus oil (1%), garlic bulb, *Zingiber officinale*, mango, termeric and lantana leaves also significantly controlled the disease. The inhibition percentages of conidial germination of *C. gloeosporioides* by different phytohormones (NAA, IAA and 2,4-D) as fungicidal treatments are given in Table 3. NAA and IAA were the most effective phytohormones against *C. gloeosporioides*, immersed after 5 to 30 minutes at 0.002-0.006% concentrations respectively. Among them, IAA has good effect against *C. gloeosporioides*. Hundred per cent germination inhibition occurred at 0.002% concentration of IAA after 30 minutes of immersion. At 0.004% concentration, IAA inhibited 100% conidial germination of *C. gloeosporioides* after 15 minutes of immersion. Hundred per cent conidial germination was inhibited at 0.005 and 0.006% of IAA, for immersion after 5-30 minutes. NAA has great effect against *C. gloeosporioides*, putting after 5-30 minutes. Hundred per cent conidial germination inhibition was observed within 30 minutes at 0.003 and 0.004% concentrations. With the increase in dose and immersion period, the inhibition rate also increased (at 0.005% of NAA within 15 minutes, 100% inhibition occurred, Table 3). At 0.006% concentration of NAA, 100% inhibition occurred after 5, 10, 15, 20, 25 and 30 minutes immersion. The growth regulator 2,4-D has good effect against *C. gloeosporioides*. Hundred per cent inhibition of conidial germination occurred at 0.005 and 0.006% concentrations of 2,4-D after 25 and 5-30 minutes of immersion respectively. Rest of the doses, have good effect against *C. gloeosporioides*. Correlation (r4) value (0.808-0.996) indicates that there was a highly significant relationship between immersion period and conidial germination inhibition, except NAA at 0.006%, IAA at 0.005% and 0.006% and 2,4-D at 0.006%. Correlation (r5) value (0.970-0.987) indicates that there was highly significant relationship between concentrations and conidial germination inhibition. Calculated F value is greater than table value in cases of immersion period and different concentrations for all types of phytohormones. There was a significant difference among phytohormone concentrations for conidial germination in given immersion period (Table 3). Sheshtawi and Kiss (1975) reported that in the laboratory MCPA and 2,4-D did not affect the development of the cereal pathogen *F. graminearum* at a concentration of 1000 ppm. Gentile and Bovio (1986) reported application of growth regulator α-naphthyl acetic acid (NAA) before inoculation of tomato plants with *F. oxysporum* f. sp. *lycopersi*, delayed expression and development of wilt symptoms.

Statistical analysis showed effective an role of selected fungicides, plant extracts and phytohormones on *C. gloeosporioides* conidial germination. Increasing the immersion period and concentration of fungicides and phytohormones has also a significant effect on conidial germination of *C. gloeosporioides*. So, this study suggests that all the tested fungicides, plant extracts and phytohormones have an antifungal effect and their application in field condition will reduce the severity of mango anthracnose disease caused by *C. gloeosporioides*.

References

Ahmed, H.U., M.M. Hossain, S.M.K. Alam, M.J. Huq and M. Hossain, 1991. Efficacy of different fungicides in controlling anthracnose and sooty mold of mango. Bangla. J. Bot., 14: 155-159.

Alam, S., M.S. Alam and F. Mahal, 1999. Growth inhibition (*in vitro*) of chilli fruit rot pathogen *Alternaria tenuis*. J. Asiat. Soc. Bangla. Sci., 25: 211-216.

Alam, S., N. Akhter, M. Begum and M.S. Alam, 2000. Effect of fungicides and plant extracts on the inhibition of *Bipolaris sorokiniana* Sacc. Rajshahi Univ. Stud. Part-B, J. Sci., 28: 15-23.

Chauhan, H.L. and H. U. Joshi, 1990. Evaluation of phyto-extracts for control mango fruit anthracnose. Botanical pesticides in integrated pest management. Proceeding of National Symposium held on January 21-22, 1990 at Central Tobacco Research Institute, Rajahmundry, 533105, India, 455-459. Rajahmundry, Indian Society of Tobacco Science.

Cochrane, V. W., 1958. Physiology of Fungi. John Wiley & Sons, Inc. New York, pp: 524.

Dubey, R.C., 1991. Fungicidal effect of essential oils of three higher plants on sclerotia of *Macrophomina phaseolina*. Indian Phytopathol., 44: 241-243.

Gentile, I. A. and M. Bovio, 1986. *Fusarium* wilt severity and ethylene evolution in tomato plants after treatment with trifluralin and naphthyl acetic acid. Zeitschrift – fur – pflanzenkra – nkheiten – und –pflanzenschuta, 93: 624-631.

Hossain, M.M., M.S. Alam and M.S. Alam, 2001. Efficacy of different fungicides in controlling purple blotch of onion seed-crop. J. Asiat. Soc. Bangla. Sci., 27: 79-84.

Mahadevan, A. and H. Sridhar, 1982. Methods in Physiological Plant Pathology. Sivakami Publications. Madras, pp: 316.

Prakash, O.M. and K. C. Srivastava, 1987. Mango diseases and their management – A World Review. Today & Tomorrow's Printers and Publishers. New Delhi, pp: 175.

Sekhare, M. J., A. Vinayagamurthy, S. Subramanian and S. Anbusm, 1989. Control of black spot of ber (*Zizyphus mauritiana*). South Indian Hort., 37: 344-345.

Sheshtawi, E. and E. Kiss, 1975. Influence of hormone type herbicides on the development of *Fusarium graminearum* Schwabe. Novenyvedelem, 11: 20-22.

Singh, H. B. and U.P. Singh, 1981. Effect of volatility of some plants extract on *Erysiphe*. Indian J. Pl. Pathol., 10: 66-67.

Singh, R.S., 1983. Plant Diseases. 5th ed. Oxford & IBH Publishing Co. New Delhi, pp: 608.

Singh, Y., R.D. Tripathi, N.N. Tripathi and S.N. Dixit, 1983. The isolation and properties of fungitoxic principle from *Zinziber officinale*. Indian J. Pl. Pathol., 1: 89-96.

Teo, C.H. and T.K. Kuch, 1982. Plant Pathology Mango (*Mangifera indica*). Annual Report of the Research Branch, Department of Agriculture, Sarawak, for the year 1982, pp: 278-279.

Snedecor, G. W. and W.G. Cochran, 1980. Statistical methods. 7th ed. Iowa State Univ. Press, Ames, Iowa, USA. pp: 507.