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USE OF BICARBONATE SALTS, FUNGICIDES AND THE BIOINSECTICIDE *Beauveria bassiana* TO SUPPRESS SOOTY MOLD DISEASE ON COTTON

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ABSTRACT

Three field trials were conducted in EL-Gemmeiza, Sirs EL-Lian and Sakha agricultural research stations in 2005 growing season to evaluate foliar application of a diverse group of compounds. The tested compounds were three bicarbonate salts (salts of sodium, potassium, and ammonium), bioinsecticide (*Beauveria bassiana*), two copper fungicides (Galben Copper and Coproxat), and a sulphur compound (that flowable micronized sulphur). Disease intensity variables (disease incidence and disease severity) and seed cotton yield were used as criteria for clarifying efficiencies of the compounds in controlling the disease. Biofly (*Beauveria bassiana*), Galben copper and Coproxat were the most effective treatments in controlling the disease in EL-Gemmeiza and Sirs EL-Lian, while bicarbonate salts were the best performing treatments in controlling the disease in Sakha. That flowable sulphur was effective in EL-Gemmeiza and Sirs EL-Lian while it was ineffective in Sakha. A highly significant ($P < 0.01$) negative correlation was observed between each of disease incidence and disease severity and seedcotton yield in EL-Gemmeiza and Sakha. While the correlation was nonsignificant in Sirs EL-Lian. In general, potassium bicarbonate, ammonium bicarbonate, and Galben were the best performing compounds in controlling the disease. This superiority was attributed to three reasons : first, potassium bicarbonate was effective in reducing SMIV in all the locations. Second, ammonium bicarbonate and Galben were effective in reducing SMIV in most of the locations. Third, the three compounds significantly increased seedcotton yield in Sakha.

INTRODUCTION

Cotton (*Gossypium barbadense*, L.) is the most important fiber crop in the world as well as in Egypt. Egypt is one of the main suppliers of extra long stable cotton which is more suitable for the manufacturing of high quality textile.

Diseases are considered among the important limiting factors of cottonseed production. Currently, sooty mold disease caused by *Cladosporium herbarum* Pers.ex.Fr. is considered as the most conspicuous, widespread and easily recognized foliar disease of cotton. Importance of the disease varies considerably from one area to another depending on cotton cultivar, cultural practices, and the prevailing environmental conditions in particular late season insect infestation (Zayed, 1997).

High population of whitefly (*Bemisia tabaci)* and cotton Aphid (*Aphis gossypii*) in the crop towards the end of the season always results in honeydew deposition on the exposed leaves and lint, which then supports the growth of the fungus under conditions of high relative humidity (Abd EL-Rehim *et al*., 1997). Elliott (1997) found that, foliar honeydew deposits from phloem feeding insects such as whiteflies and Aphids are often colonized by sooty mold fungus. Although, this fungus is not considered as a pathogen in the strict sense, it may impact photosynthesis in a negative manner. Studies were conducted to determine the impact of epiphytic colonizers on photosynthesis of cotton. Photosynthesis rates were inversely proportional to sooty mold levels with an 80% reduction in photosynthesis at the highest level of colonization. Stomatal conductance was also reduced at higher sooty mold levels. Internal CO_2 level was slightly higher in sooty mold colonized levels but was not proportional to the degree of colonization.

Fungicides are widely used for controlling *Cladosporium spp.* on many crops. For example, leaf spot caused by *Cladosporium humile* on cuttings of *Populus ciliata* was controlled with the fungicides Dithane M-45 (Mancozeb) at 0.35 %, Bavistin (Carbindazim) at 10.05 and Bitox (copper oxychloride) at 0.2-0.3 %. These fungicides gave disease control of 66.7 %, 51.4-59.9 %, and 33-48.7 % respectively (Khan *et al.*, 1990). Cal *et al.*,(1992) reported that population of *Cladosporium sp.* on peach twigs was considerably depressed by fungicides applied singly (Copper oxychloride and Thiram) or in combination (Captan, Dinocap and Benomyl). Wang and Wan (1992) stated that sooty blotch of rice was observed in Ningxia in China in 1991, and the pathogen was identified as *Cladosporium herbarum*. Tricyclazole, Carbendazol and streptomycin provided 45.3-60.8% disease control. Ziv (1992) found that, sodium, potassium and ammonium bicarbonates alone and in combination with sun spray ultra-fine spray oil (SS) were effective in controlling powdery mildew, gummy stem blight, Alternaria leaf blight and Ulocladium leaf spot on cucurbit. He also stated that all the three bicarbonates (1 % w/v) plus SS oil (1% w/v) and especially ammonium bicarbonate plus SS provided control of gummy stem blight and Alternaria leaf blight of muskmelon and Ulocladium leaf spot of cucumber in greenhouse trails. In vitro studies, showed that bicarbonate salts inhibited growth of these organisms, ammonium bicarbonate was most inhibitory. Sodium, potassium and ammonium chloride salts were least inhibitory. Horst *et al.* (1992) stated that, powdery mildew caused by *Diplocarpan rosae*, were significantly controlled by weekly sprays of 0.06 M aqueous solution of sodium bicarbonate plus 1.0 % (w/v) SS on *Rosa spp*. Katole *et al.* (1994) reported that, copper oxychloride alone did not gave effective control of sooty mold on *Nagpur mandarine* in India. But, when insecticides applied with copper oxychloride gave better control of sooty mold. Zayed (1997) found that, the fungicide Dithane M-45, Copproxat and the insecticidal mixture of Applaued and Nuvacron gave the best control of sooty mold disease on cotton. Pitts *et al.*, (2002) stated that, the Captan 50 wp. 5 lb/A and Captan 3 lb/A + sulphur 80 % 5.5 lb/A treatments had fewer peach fruits with scab (*Cladosporium carpophilum*) and higher levels of marketable fruit than the sulphur 80 % at 9 lb/A treatment.

The objective of this study was to estimate the effects of seven compounds on disease intensity variables of sooty mold on cotton and on seedcotton yield under filed conditions in three locations.

MATERIALS AND METHODS

Fungal isolation

Isolates of *Cladosporium herbarum* Pers.ex.Fr. (Table 1) used in this study were isolated, purified and identified at cotton pathology Lab., Plant Path. Res. Inst., Agric. Res. Cent., Giza.

Table (1) : Sources and geographic origins of *Cladosporium herbarum* isolates used in artificial inoculation of field trails.

Isolate No.	Cultivar	Geographic origin	Source
1	Unknown	Unknown	Carpellary wall (pericarp)
2	Giza 75	Minya EL-Kamh-Sharqiya	Leaf
3	Dendera	Sohag-Sohag	Leaf
4	Giza 81	Hihya-Sharqiya	Leaf
5	Giza 75	Fakkous-Sharqiya	Leaf
6	------	Giza-Giza	Air
7	Giza 75	EL-Mansoura-Dakahlia	Carpellary wall (pericarp)

Preparation of inoculum used in artificial inoculation of field plots :

Substrate for growth of fungal isolates used in artificial inoculation of field plots, was prepared in 500 ml glass bottles, each bottle contained 50 g of sorghum grains an 40 ml of tap water, contents of bottle were autoclaved for 30 min. Fungal inoculum, taken from one-week-old culture, was aseptically introduced into the bottle and allowed to colonize sorghum for 3 weeks. Contents of each bottle was blended with equal amount of water and filtered through cheesecloth. The obtained suspension which contained conidiospores and mycelium fragments was applied as foliar sprays on cotton plants.

Field evaluation of fungicides and assessment of disease intensity variables :

Experiments were conducted at EL-Gemmeiza, Sirs EL-Lian, and Sakha Agricultural Research Stations in 2005. Each experiment was designed as a randomized complete block of four replicates, each replicate consisted of six ridges, 5 meter length and 70 cm width. Each row included 25 hills, each containing 2 seedlings of cultivar 89 after thinning.

Planting dates were 25 March in EL-Gemmeiza, 30 March in Sirs EL-Lian and 4 April in Sakha. Field plots were artificially inoculated on 2 August in EL-Gemmeiza, 5 August in Sakha and 10 August in Sirs EL-Lian. Sooty mold was allowed to developing for about two weeks before the initial fungicide application. Foliar sprays were applied at the recommended rates (Table 2) on 16 August and 1 September in EL-Gemmeiza, 19 August and 3 September in Sirs EL-Lian and on 24 August and on 8 September in Sakha. Disease incidence (DI) and disease severity (DS) were rated visually after two weeks from the second spray. DI was measured as percentage of infected leaves in a random sample of 100 leaves/plot. DS was assessed on a 5 grade scale (from 0 to 4) where grade 0 indicates healthy leaf, grade 1 = sooty mold on 25 % of the total area, grade 2 = sooty mold on 50 % of the total area, grade 3 = sooty mold on 75 % of the total area and grade 4 = sooty mold on 100 % of the total area (Zayed, 1997). Seedcotton yield (cotton seed and lint before ginning) was picked on 15-30 October at each site.

Table (2) : Fungicides, biocides and chemical compounds used for control of sooty mold of cotton under field conditions in EL-Gemmeiza, Sirs EL-Lian and Sakha in 2005.

Fungicide [a]	Rate (per 100 liter of water)	Active ingredient [b]	Formulation [c]
Sodium bicarbonate	1000 g	$NaHCO_3$	Sp
Potassium bicarbonate	1000 g	$KHCO_3$	Sp
Ammonium bicarbonate	1000 g	NH_4HCO_3	Sp
Biofly (*Beauveria bassiana*)	100 cm	3×10^7 conidia/ml	FL
Galben copper	150 g	11% Benalaxyl + 35% copper oxychloride	Wp
Copproxat	300 ml	19% Metalic copper	FL
That flowable sulfur	250 ml	52% sulfur	FL

[a] Trade name
[b] Common name
[c] Sp = Soluble powder
Wp = Wettable powder
FL = Flowable liquid

Statistical analysis of the data :

Analysis of variance (ANOVA) of the data was performed with MSTAT-C statistical package (A micro computer program for the design, management and analysis of Agronomic Research Experiments, Michigan State Univ., USA). Least Significant Differences (LSD) was used to compare treatment means. Correlation and regression analyses were performed with a computerized program.

RESULTS AND DISCUSSION

Data in Tables (3-5) showed that in general all the tested treatments reduced disease incidence (DI) and disease severity (DS) of sooty mold disease on cotton compared with control; however, they showed different levels of efficiency from one location to another, which may indicate the occurrence of fungicide x environment interaction. Thus, Galben copper, Biofly (*Beauveria bassiana*) and Copproxat were the most effective treatments in reducing DI and DS of the disease in EL-Gemmeiza and Sers EL-Lian while the bicarbonate salts were the best performing compounds in Sakha. That flowable sulfur was effective in EL-Gemmeiza and Sirs EL-Lian, while it was ineffective in Sakha. The efficiency of Galben copper and Copproxat in controlling sooty mold disease on cotton is in concert with the results of other workers. For example, spraying with Cu preparation is recommended for controlling melanosis caused by *Cladosporium herbarum* on apple (Smolak, 1960). Cal *et al.* (1992) found that the population of *Cladosporium spp.* on peach twigs was considerably depressed by copper oxychloride. Zayed (1997) found that, the fungicide Dithane M-45, Copproxat and the insecticidal mixture of Applaud and Nuvacron gave the best control of sooty mold disease on cotton. It is worthy of mention that Galben copper and Copproxat (metallic copper) exert their fungicide effect by metabolizing to isothiocyanate radical (- N = C = S), which inactivates the sulfhydry group (-

SH) in amino acids and enzymes within the pathogen cells and thereby inhibits the production and function of these compounds (Agrious, 1988).

It is believed that bicarbonate salts exert their antifungal effects by inhibiting conidial formation and germination (Horst *et al.*, 1992).

That flowable sulfur is a surface protectant that suppresses fungal growth and sporulation either by direct contact or vapor phase activity (Seem *et al.*, 1981).

Table (3) : Effects of foliar applications with bicarbonate salts, biofly and fungicides on disease intensity variables of sooty mold of cotton and on seedcotton yield in EL-Gemmeiza in 2005.

Treatments	Disease incidence [b]	Disease severity [d]	Seedcotton yield (kntar/feddan)
Sodium bicarbonate	65.5 [c]	1.90	8.45
Potassium bicarbonate	69.0	2.25	8.38
Ammonium bicarbonate	72.5	2.50	8.19
Biofly (*Beauveria bassiana*)	62.0	1.55	8.59
Galben copper	55.0	1.15	8.89
Copproxat	58.0	1.38	8.76
That flowable sulfur	64.0	1.70	8.51
Control [a]	85.0	2.85	8.06
L.S.D ($P \leq 0.05$)	9.18	0.54	N.S
($P \leq 0.01$)	12.49	0.74	N.S

[a] Plants were sprayed with water

[b] Disease incidence (percentage of infected leaves in a random sample of 100 leaves/plot).

[c] Mean of four replications.

[d] Disease severity was assessed in a random sample of 100 leaves/plot on a 5 grade scale (from 0 – 4) where grade 0 indicated healthy leaf and grade 4 the most infected one.

Table (4) : Effects of foliar application with bicarbonate salts, biofly and fungicides on disease intensity variables of sooty mold of cotton and on seedcotton yield in Sirs EL-Lian in 2005.

Treatments	Disease incidence [b]	Disease severity [d]	Seedcotton yield (kntar/feddan)
Sodium bicarbonate	24.50 [c]	2.42	8.55
Potassium bicarbonate	23.75	2.35	7.77
Ammonium bicarbonate	27.75	2.78	7.57
Biofly (*Beauveria bassiana*)	17.00	1.80	6.73
Galben copper	25.00	2.58	7.00
Copproxat	22.00	2.20	6.38
That flowable sulfur	24.75	2.30	8.80
Control [a]	35.00	3.50	7.53
L.S.D ($P \leq 0.05$)	3.8	0.29	N.S
($P \leq 0.01$)	5.1	0.39	N.S

[a] Plants were sprayed with water

[b] Disease incidence (percentage of infected leaves in a random sample of 100 leaves/plot).

[c] Mean of four replications.

[d] Disease severity was assessed in a random sample of 100 leaves/plot on a 5 grade scale (from 0 – 4) where grade 0 indicated healthy leaf and grade 4 the most infected one.

High population of whitefly (*Bemisia tabaci*) and cotton Aphid (*Aphids gossypii*) in cotton towards the end of the season always results in honeydew deposition on the exposed leaves and lint, which then supports the growth of *Cladosporium herbarum* under conditions of high relative humidity (Abd EL-Rehim *et al.*, 1997 and Zayed, 1997). Thus, it can be reasonably assumed that the efficiency of the biofly (*Beauveria bassiana*) in controlling the disease is due to its efficiency in reducing whitefly and Aphid populations.

Table (5) : Effects of foliar application with bicarbonate salts, biofly and fungicides on disease intensity variables of sooty mold of cotton and on seedcotton yield in Sakha in 2005.

Treatments	**Disease incidence** [b]	**Disease severity** [d]	**Seedcotton yield (kntar/feddan)**
Sodium bicarbonate	50.25 [c]	1.08	4.38
Potassium bicarbonate	37.50	0.70	5.68
Ammonium bicarbonate	40.50	0.83	4.92
Biofly (*Beauveria bassiana*)	61.50	1.50	3.65
Galben copper	55.00	0.95	5.00
Copproxat	60.50	1.08	3.38
That flowable sulfur	59.50	1.35	4.13
Control [a]	69.00	1.40	3.55
L.S.D ($P \leq 0.05$)	17.05	0.42	1.29
($P \leq 0.01$)	N.S	0.58	N.S

[a] Plants were sprayed with water

[b] Disease incidence (percentage of infected leaves in a random sample of 100 leaves/plot).

[c] Mean of four replications.

[d] Disease severity was assessed in a random sample of 100 leaves/plot on a 5 grade scale (from 0 – 4) where grade 0 indicated healthy leaf and grade 4 the most infected one.

Data in Tables 6 and 7 and Figs. 1 and 2 showed highly significant negative correlations between seedcotton yield and sooty mold intensity variables (SMIV) in EL-Gemmeiza and Sakha Agric. Res. Stations. Thus, sooty mold accounted for 65 to 97 % of the total variation in seedcotton yield. The most likely explanation for the negative association between seedcotton yield and SMIV is that sooty mold may inhibit physiological processes, which ultimately reduced yield. This interpretation is in agreement with the findings of other workers. For example, net CO_2 assimilation, conductance to water vapor, transpiration rate and chlorophyll content of pecan leaves were reduced in a linear or curvilinear manner with an increase in scab infection caused by *Cladosporium caryigenum* (Gould *et al.*, 1996). Elliott (1997) found that, foliar honeydew deposits from phloem feeding insects such as whiteflies and Aphids are often colonized by sooty mold fungus. Although, this fungus is not a pathogen in the strict sense, it may impact photosynthesis in a negative manner. In general, potassium bicarbonate, ammonium bicarbonate, and Galben were the best performing compounds in controlling the disease. This superiority was attributed to three reasons : first, potassium bicarbonate was effective in reducing SMIV in all the locations. Second, ammonium bicarbonate and Galben were effective in reducing SMIV in most of the locations. Third, the three compounds significantly increased seedcotton yield in Sakha.

Table (6) : Correlation between disease intensity variables and seed cotton yield.

Location	Disease intensity variables	Correlation with seedcotton yield
EL-Gemmeiza	Disease incidence	- 0.938** [a]
	Disease severity	- 0.984**
Sirs EL-Lian	Disease incidence	0.29
	Disease severity	0.17
Sakha	Disease incidence	- 0.807**
	Disease severity	- 0.895**

[a] Linear correlation coefficient (r) is significant at $P \leq 0.01$ (**).

Table (7) : Regression equations to describe the effects of disease incidence and disease severity (x) on seedcotton yield (y) under field conditions in 2005.

Location [a]	Indepent variable (x)	Regression equation	R_2 [b]	F. value	P > F
EL-Gemmeiza	Disease incidence [c]	$y^d = 10.29 - 0.027\,x$	0.879	43.72	0.001
	Disease severity [e]	$y = 9.36 - 0.465\,x$	0.967	178.11	0.000
Sakha	Disease incidence	$y = 7.44 - 0.057\,x$	0.651	11.20	0.015
	Disease severity	$y = 7.03 - 2.38\,x$	0.802	24.25	0.003

[a] No regression model was constructed for Sirs EL-Lian because lack of significant correlation between yield and disease intensity variables (disease incidence and disease severity).

[b] Coefficient of determination.

[c] Disease incidence is the percentage of infected leaves in a random sample of 100 leaves/plot.

[d] Seedcotton yield in kentar/feddan.

[e] Disease severity on a 5-grade scale (from 0-4) where grade 0 indicates the healthy leaves and grade 4 indicates the most infected ones.

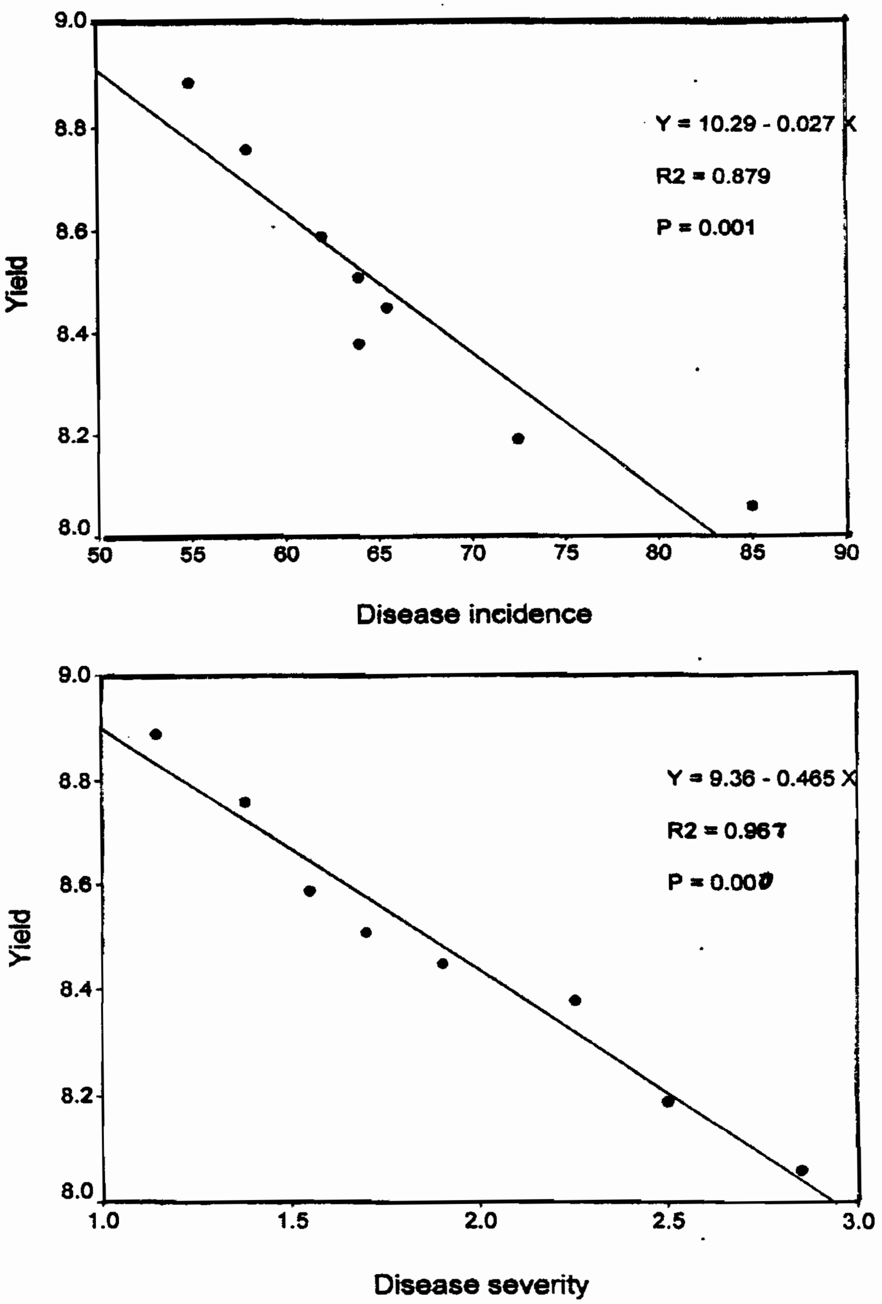

Fig. 1: Regression equations that describe the effects of disease incidence and disease severity on seedcotton yield under field conditions in EL-Gemmeiza in 2005.

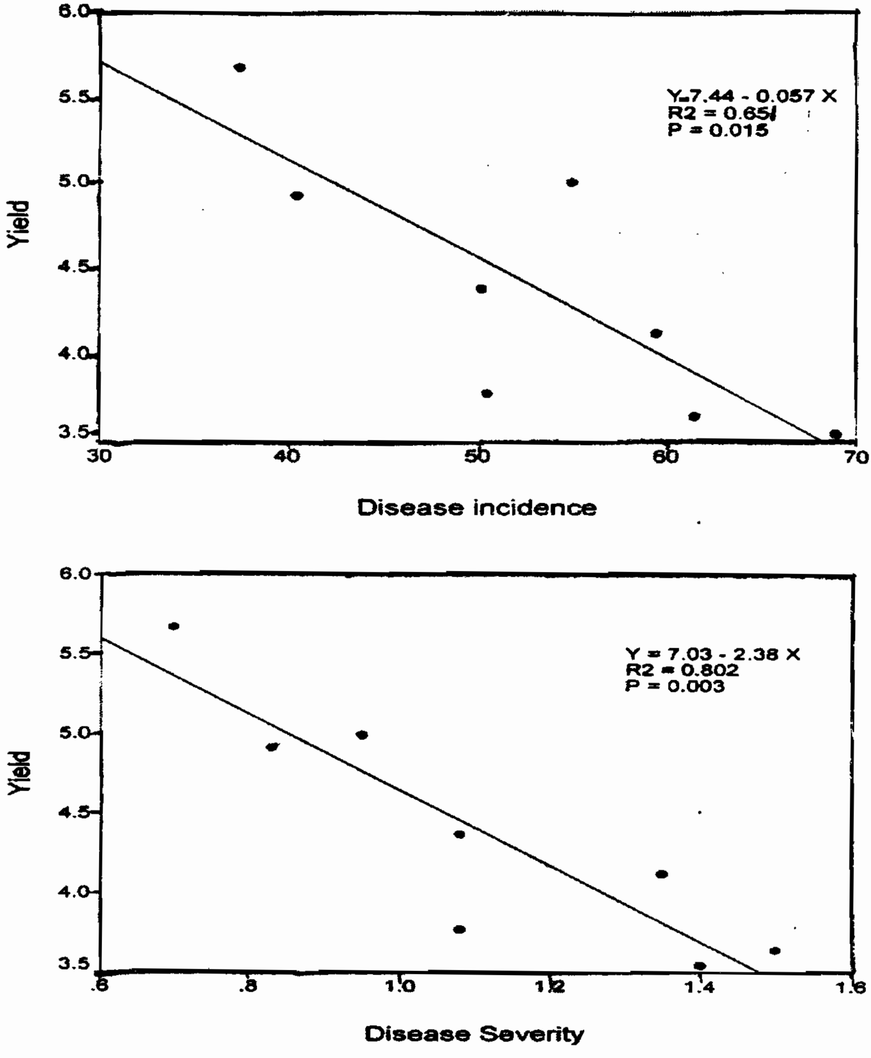

Fig. 2: Regression equations that describe the effects of disease incidence and disease severity on seedcotton yield under field conditions in Sakha in 2005.

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إستخدام أملاح البيكربونات والمبيدات الفطرية والمبيد الحشرى الحيوى (بوفاريا باسيانا) لمقاومة مرض العفن الأسود في القطن

شوقى محمد المتولى زايد ، معوض رجب عمر و عبد الرحيم محمد أحمد السمواتى

معهد بحوث أمراض النبات – مركز البحوث الزراعية – الجيزة – مصر

أجريت ثلاث تجارب حقلية عام ٢٠٠٥ بمحطات بحوث الجميزة وسرس الليان وسخا ، لتقييم فعالية الرش بمجموعة متنوعة من المركبات في مقاومة مرض العفن الأسود في القطن. إشتملت المركبات المقيمة على ٣ أملاح بيكربونات (أملاح صوديوم وبوتاسيوم وأمونيوم) والمبيد الحشرى الحيوى بيوفلاى (فطر بوفاريا باسيانا) ومركبين نحاسيين (جالبين نحاس وكوبروكسات) ومركب كبريتى (ذات فلابل سلفر). إستعملت المتغيرات الدألة على كثافة المرض (حدوث المرض وشدة المرض) ومحصول القطن الزهر كمعايير لتقييم أداء المركبات المختبرة. أظهرت الدراسة أن أفضل المبيدات أداءً في مقاومة المرض كانت بيوفلاى وجالبين نحاس وكوبروكسات وذلك في كل من الجميزة وسرس الليان بينما كانت أملاح البيكربونات الأفضل أداءً في سخا. لوحظت إرتباطات سالبة عالية المعنوية بين المتغيرات الدالة على كثافة المرض والمحصول في كل من الجميزة وسخا في حين كانت الإرتباطات غير معنوية في سرس الليان. وعموماً أظهرت الدراسة أن أفضل المركبات أداءً في مقاومة المرض هي : بيكربونات البوتاسيوم وبيكربونات الأمونيوم وجالبين نحاس ، ويعزى هذا التفوق إلى تفرد هذه المركبات بمجموعة من المزايا هي : (١) بيكربونات البوتاسيوم كانت فعالة في التقليل من المتغيرات الدالة على كثافة المرض في جميع المواقع ، (٢) بيكربونات الأمونيوم وجالبين نحاس أظهر فاعلية في التقليل من المتغيرات الدالة على كثافة المرض في أغلب المواقع ، (٣) المركبات الثلاثة أحدثت زيادات معنوية في محصول القطن المزهر في سخا.