

BIOEFFICACY OF BOTANICALS AGAINST ALTERNARIA LEAF BLIGHT OF MUSTARD UNDER FIELD CONDITION

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KEYWORDS

Mustard
Alternaria blight
Botanicals
Doses
Seed yield

Received on :

22.03.2013

Accepted on :

06.05.2013

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ABSTRACT

Aqueous extract of three botanicals as leaves of neem (*Azadirachta indica*), bulbs of garlic (*Allium sativum*) and rhizome of ginger (*Zingiber officinale*) at four different doses (5, 10, 15, 20%) were evaluated against Alternaria leaf blight of mustard under field condition. The disease was adequately managed by the application of these three botanicals irrespective of their doses in comparison to untreated control. Two years data revealed that spraying of neem leaf extract @15% was more effective against this disease as well as increased the seed yield of mustard. Seed yield was significantly highest ($p < 0.05$) on application of neem leaf extract @15% over other treatments (1403.83 kg/ha) where as in check had only 820.33kg/ha. Garlic bulb extract@10% also gave better yield (1366.17kg/ha) which is similar to that of 10% of neem leaf extract (1383.00kg/ha). Cost benefit ratio was also highest (1:2.5) at 15% neem leaf extract which was similar to that of 10% of the same botanicals (1:2.0) and 10% garlic bulb extract (1:1.9). It indicating that the spraying of neem leaf extract on mustard was effective against Alternaria blight resulting superior performances in seed yield for consecutive two years.

INTRODUCTION

Indian mustard [*Brassica juncea* (L). Czern and Cross] is the principle rabi oilseed crop in India which covers 22% area and contributes 25% of production of total oil seed crops. Alternaria leaf blight (ALB) caused by *Alternaria brassicae* and *Alternaria brassicicola* has been reported from all the continents of the world and caused upto 10-70% yield loss (Kumar, 1997). In addition to direct losses in yield, the disease adversely affect the seed quality by reducing seed size, seed discolouration and reduction in oil content (Rajendra and Lallu, 2006). Different chemicals including systemic fungicides have been used for management of this disease (Chattapadhyay and Vhunia, 2003). However increase environmental pollution and present day public perception on pesticide contaminants of foods specially the edible oils, development of alternate economical and ecofriendly approaches for disease management is needed several plant products are known to have antifungal activities which are environmentally safe and non phytotoxic also (Bisht and Khulbe 1995; Meena *et al.*, 2004). The damage in these chemicals was brought an awareness to find out other alternatives like eco-friendly management with the framework of IDM without affecting our precious eco-system (Mukhopadhyay, 1994) Currently studies pertaining to the use of botanicals in management of pathogens and related diseases are highly focused (Koche, 2013; Toppo, 2013; Mathad, 2013; Mathad, 2013; Mahapatra, 2013; Bisht, 2013). So, the present study was conducted to generate information on effect of the aqueous extract of different botanicals (leaves of neem, bulbs of garlic and rhizomes of ginger) and their effective

doses against this destructive disease of mustard.

MATERIALS AND METHODS

A field experiment was conducted for two consecutive years (2008-09 and 2009-10) during rabi season at University Instructional Farm, Jaguli, Bidhan Chandra Krishi Viswavidyala under natural field condition. The variety, Binoy (B-9) was sown in randomised block design with thirteen treatments including check (untreated control). The plot size was 5x5 sq.m. and replicated thrice for each treatment. Recommended agronomic practices were followed to raise the crop. Three botanicals namely leaves of neem (*Azadirachta indica*), bulbs of garlic (*Allium sativum*) and rhizome of ginger (*Zingiber officinale*) with four different doses (5, 10, 15, 20%) of each botanical were sprayed four times at ten days interval starting from initial appearance of disease (40 days after sowing). One check (untreated control) was also maintained receiving water spray. Aqueous extract of these three botanicals (1% w/v) were prepared by mixing 100 g fresh leaves, bulbs and rhizomes of respective plants with 100 mL of sterile distilled water and crushing individually warring blender. Extract was filtered through double layer mushlin cloth before use. The filtrat thus obtained was considered as 100% plant extract. Similarly, different doses of aqueous extract (5, 10, 15, 20%) were prepared individually according to the treatments. the different doses of aquese extract of these three botanicals were selected as per doses used by different scientist on other crops *in vitro* condition (Patil *et al.*, 2003) on early blight of tomato and extract of dried roots of *Acorus calamus* against *alternaria solani* of tomato (Vadivel and Ebelezer, 2006). The

observations of percent leaf infection, disease severity were taken from 40 DAS to 100 DAS, at an interval of 10 days. The number of spots / siliqua, the percent of siliqua infection and the data on yield parameters were taken at harvest. The percent of infection was assessed as no. of leaves infected/10 plants and disease severity was assessed as the average disease index of leaves. Percent *Alternaria* blight severity was recorded on leaves and pods at 10 days interval following scale 0 – 5 disease rating scale of Sharma and Kolte (1994), where, 0 = no visible symptoms, 1 = 1 – 10%, 2 = 11 – 25%, 3 = 26 – 50%, 4 = 51 – 75% and 5 = >75% leaf area infected. Evaluation was done on randomly selected plant in each replicated block for measuring the disease progress.

$$\text{Percent Disease Index (PDI)} = \frac{\text{Sum of all numerical ratings}}{\text{Total number of leaf observed} \times \text{maximum rating}} \times 100$$

Disease severity (AUDPC) calculated as per Wilcoxon *et al.*, 1975. The formula was used as follows: where, $AUDPC = [(Y_{i+1} + y_i) / 2 (X_{i+1} - X_i)]$

Y_i = severity at 1st observation, X_i = Time (days) at first observation, N = Total number of observation.

RESULTS AND DISCUSSION

The results showed that the disease and yield parameters were different on different years of study due to change in environment in field. So, the pooled data of two years (2008-09 and 2009-10) on all the parameters were presented and discussed.

Disease parameters

The two years (2008-09 and 2009-10) pooled mean showed that all the three botanicals (Neem leaf extract, Ginger rhizome extract and Garlic bulb extract) significantly ($p < 0.05$) reduced the percent leaf infection in comparison to untreated control. Minimum percent in leaf infection was recorded in Garlic Neem leaf extract @ 15% (20.21%) followed by garlic leaf extract @ 20% (20.22%) and 15% (20.78%). And the most least effective botanical on reducing the percent leaf infection was Ginger rhizome extract when applied in plots at @ 5% (28.41%) dose.

In case of disease severity (AUDPC), the three botanicals and their different doses showed different disease severity in two different years and the pooled mean. In the year of 2008-09, minimum disease severity (AUDPC) was recorded in Garlic bulb extract @ 20% (21.09) which was statistically at par on Neem leaf extract @ 15% (20.21) and maximum disease severity (AUDPC) was recorded in Ginger rhizome extract @ 5% (26.30) which was at par with 20% of this botanical (26.06). In the year of 2009-10, the minimum disease severity was recorded on 15% Garlic bulb extract (19.07) followed by 15% and 20% Neem leaf extract (19.60 and 19.76 respectively). No significant ($p < 0.05$) difference in decreasing the leaf blight severity was noticed among the treatments like 10% Garlic bulb extract (20.13) and 10% Neem leaf extract (20.10). Two years pooled mean showed minimum disease severity (AUDPC) was in 15% Neem leaf extract (19.90) statistically at

par with 15% doses of Garlic bulb extract (19.92) and followed by 20% of Neem leaf extract (20.26). It was also observed that 15% dose of Neem leaf extract (19.90) and Garlic bulb extract (19.92) and 20% dose of Neem leaf extract showed no significant differences among themselves in reducing (20.26%) the disease severity (Table1).

The percent of siliqua infection in consecutive two years and in pooled mean showed that the three botanicals and their four doses produced different percentage of siliqua infection significantly ($p < 0.05$). In the year 2008-09, minimum percent of siliqua infection was recorded on 15% Neem leaf extract (15.24%) statistically at par on Garlic bulb extract 15% (15.85%). Neem leaf extract@10% (16.40%) and Garlic bulb extract@10% (16.83%) showed no significant difference in between them in respect to decrease in percent of siliqua infection. In the year 2009-10, the minimum percent of siliqua infection was observed on 15% Neem leaf extract (18.25%) at par with 10% (18.30%) followed by 15% of Garlic bulb extract (18.53%). The two years pooled mean showed the minimum percent of siliqua infection on 15% dose of Neem leaf extract (16.74%) at par with 15% dose of Garlic bulb extract (17.19%). It was also observed that 10% Neem leaf extract (17.35%) and 10% Garlic bulb extract (17.84%) produced minimum percent of siliqua infection (Table2).

The minimum number of spots per siliqua was showed on 15% of Neem leaf extract (8.15) at par with 10% and 20% dose of this botanical (8.23 and 8.35 respectively) and also 10%, 15% and 20% doses of Garlic bulb extract (8.29, 8.65 and 8.23 respectively) in the year 2008-09. Similarly, 2009-10, minimum number of spots per siliqua was recorded on 15% dose of Neem leaf extract (8.40) similar with 10% and 20% dose of this botanical (8.47 and 9.05 respectively). 10% and 20% of Garlic bulb extract also produced minimum number of spots per siliqua (8.50, 8.73 respectively) which was at par with 10 to 20% of Neem leaf extract). The two years pooled mean also showed minimum number of spots per siliqua was recorded on 15% doses of Neem leaf extract (8.27) statistically similar with 10% and 20% of the same botanical (8.35 and 8.70 respectively). Similarly, Garlic bulb extract@ 10% to 20% dose of produced minimum number of spots per siliqua statistically at par with (8.51, 8.78 and 8.36 respectively), Neem leaf extract of same doses (Table2).

Yield parameters

The yield parameters e.g. number of siliqua per plant also noted different results in different years. In the year 2008-09, maximum number of siliqua per plant was recorded on 10% Garlic bulb extract (90.57) followed by Neem leaf extract at 10% (88.62). Two different doses of Neem leaf extract showed that 10%, 15% and 20% dose showed no significant difference ($p < 0.05$) in between them in increase the number of siliqua per plant (88.62, 87.28 and 87.12 respectively). Similarly, 5%, 15% and 20% doses of Garlic bulb extract also increased the number of siliqua per plant but their differences were not statistically significant ($p < 0.05$) (86.25, 86.42 and 85.37 respectively). Minimum number of siliqua per plant was harvested on 5% dose of Ginger rhizome extract (81.73) followed by 10% and 20% dose of the same botanical (84.33 and 84.69 respectively) (Table 3). In the year 2009-10, maximum increase in number of siliqua per plant was obtained

Table 1: Different doses of three botanical on Percent of leaf infection and Disease severity (AUDPC) due to ALB of mustard for two consecutive years and pooled mean

Botanicals	Dose (%)	Percent of leaf infection			Disease severity (AUDPC)		
		2008-09	2009-10	Pooled	2008-09	2009-10	Pooled
Neem leaf extract	5	20.97(27.25)	24.84(29.89)	22.90 (28.57)	22.79	26.93	24.86
	10	21.38(27.54)	21.65(27.73)	21.51 (27.63)	21.26	20.10	20.68
	15	19.59(26.27)	21.03(27.29)	20.31 (26.78)	20.21	19.60	19.90
	20	19.19(25.98)	21.26(27.46)	20.22 (26.72)	20.76	19.76	20.26
Ginger rhizome extract	5	27.16(31.41)	29.66(33.00)	28.41 (32.20)	26.30	29.94	28.12
	10	23.23(28.82)	26.04(30.68)	24.63 (29.75)	25.01	23.23	24.12
	15	23.33(28.88)	26.25(30.82)	24.79 (29.85)	25.13	22.94	24.03
	20	20.94(27.23)	28.06(31.98)	24.50 (29.60)	26.06	26.87	26.46
Garlic bulb extract	5	20.80(27.12)	25.21(30.14)	23.00 (28.63)	23.14	23.05	23.09
	10	20.84(27.16)	22.16(28.08)	21.50 (27.62)	21.66	20.13	20.89
	15	19.22(26.00)	21.09(27.34)	20.15 (26.67)	20.78	19.07	19.92
	20	19.53(26.23)	22.10(28.04)	20.81 (27.13)	21.09	20.34	20.71
CONTROL	0	37.47(37.74)	39.93(39.19)	38.70 (38.46)	33.98	32.40	33.19
		Sem CD	Sem CD	Sem CD	Sem CD	Sem CD	Sem CD
		(±) (5%)	(±) (5%)	(±) (5%)	(±) (5%)	(±) (5%)	(±) (5%)
Botanicals		0.16 0.47	0.12 0.36	0.12 0.35	0.11 0.31	0.10 0.30	0.1 0.28
Dose		0.19 0.54	0.14 0.41	0.14 0.41	0.12 0.36	0.12 0.35	0.11 0.32
Botanicals x Dose		0.32 0.94	0.24 0.71	0.24 0.71	0.22 0.63	0.21 0.60	0.19 0.56
Botanicals x Control		0.29 0.61	0.39 0.81	0.25 0.52	0.25 0.52	0.30 0.61	0.2 0.41

(Figures within the paranthesis are angular transformed value)

Table 2: Different doses of three botanical on Percent of siliqua infection and number of spots per siliqua due to ALB of mustard for two consecutive years and pooled mean

Botanicals	Dose (%)	Percent of siliqua infection			Number of spots per siliqua		
		2008-09	2009-10	Pooled	2008-09	2009-10	Pooled
Neem leaf extract	5	20.04(26.60)	21.62(27.71)	20.83 (27.15)	9.78	10.40	10.09
	10	16.40(23.89)	18.30(25.33)	17.35 (24.61)	8.23	8.47	8.35
	15	15.24(22.98)	18.25(25.29)	16.74 (24.13)	8.15	8.40	8.27
	20	17.32(24.59)	19.65(26.31)	18.48 (25.45)	8.35	9.05	8.70
Ginger rhizome extract	5	22.99(28.65)	24.79(29.86)	23.89 (29.25)	11.54	12.27	11.90
	10	23.14(28.76)	20.83(27.16)	21.98 (27.96)	10.38	11.23	10.80
	15	20.43(26.87)	20.01(26.57)	20.22 (26.72)	10.51	11.01	10.76
	20	25.85(30.56)	23.91(29.27)	24.88 (29.91)	11.99	12.57	12.28
Garlic bulb extract	5	20.13(26.66)	22.70(28.45)	21.41 (27.55)	9.82	10.43	10.12
	10	16.83(24.22)	18.86(25.74)	17.84 (24.98)	8.29	8.73	8.51
	15	15.85(23.46)	18.53(25.49)	17.19 (24.47)	8.69	8.88	8.78
	20	17.06(24.39)	19.12(25.93)	18.09 (25.16)	8.23	8.50	8.36
CONTROL	0	32.79(34.93)	31.69(34.26)	32.24 (34.59)	15.91	16.45	16.18
		Sem CD	Sem CD	Sem CD	Sem CD	Sem CD	Sem CD
		(±) (5%)	(±) (5%)	(±) (5%)	(±) (5%)	(±) (5%)	(±) (5%)
Botanicals		0.13 0.38	0.09 0.25	0.08 0.22	0.15 0.44	0.29 0.84	0.12 0.36
Dose		0.15 0.44	0.10 0.29	0.09 0.25	0.17 0.51	0.33 0.97	0.14 0.41
Botanicals x Dose		0.26 0.77	0.17 0.50	0.15 0.44	0.30 0.87	0.58 1.68	0.25 0.72
Botanicals x Control		0.73 1.51	0.22 0.46	0.16 0.32	0.29 0.59	0.96 1.97	0.26 0.53

(Figures within the paranthesis are angular transformed value)

from the plots sprayed with 15% Neem leaf extract (88.20) at par with 10% Garlic bulb extract (87.92). 10% Minimum increase in number of siliqua per plant was harvested on 20% Garlic bulb extract (74.11) statistically at par on 5% and 20% Ginger rhizome extract (75.49 and 78.61 respectively). The two year pooled mean also showed maximum increase in number of siliqua per plant on 10% Garlic bulb extract (89.24) similar with 10%, 15%, 20% doses of neem leaf extract (86.27, 87.74 and 85.57 respectively). Similarly, 10%, 15%, 20% doses of Ginger rhizome extract (84.50, 84.33 and 81.65 respectively) and 5% and 15% doses of Garlic bulb extract (84.35 and 84.16 respectively) showed no significant difference ($p < 0.05$) among themselves in increase the number of siliqua per plant. Minimum increase in siliqua per plant was noted on 5% Ginger rhizome extract (78.61) at par on 20% Garlic

bulb extract (79.74) (Table 3).

Application of three botanicals and their respective four doses ultimately reflected on seed yield (kg ha^{-1}) of mustard. Three botanicals, four doses and their interaction were statistically significant among themselves in respect to seed yield (kg ha^{-1}). This type of result was noticed in both the two years and in pooled mean. In the year 2008-09, maximum seed yield was obtained 15% Neem leaf extract ($1464.33 \text{ kg ha}^{-1}$) statistically at par with 10% of this botanicals ($1430.67 \text{ kg ha}^{-1}$ and $1411.33 \text{ kg ha}^{-1}$ respectively) dose of Garlic bulb extract ($1425.67 \text{ kg ha}^{-1}$). No significant difference in seed yield (kg ha^{-1}) was noticed on 15% dose of Garlic bulb extract ($1308.67 \text{ kg ha}^{-1}$), 20% dose of Ginger rhizome extract ($1321.33 \text{ kg ha}^{-1}$). In the year 2009-10, showed maximum seed yield on 15% dose of Neem leaf extract ($1343.33 \text{ kg ha}^{-1}$) similar with 10% ($1335.33 \text{ kg ha}^{-1}$)

Table 3: Different doses of three botanical on Number of siliqua per plant and yield (kg ha⁻¹) due to ALB of mustard for two consecutive years and pooled mean

Botanicals	Dose (%)	Number of siliqua per plant			Yield (kg ha ⁻¹)			Cost:benefit ratio
		2008-09	2009-10	Pooled	2008-09	2009-10	Pooled	
Neem leaf extract	5	86.08	81.38	83.73	1219.00	1112.33	1165.66	1: 0.5
	10	88.62	83.92	86.27	1430.67	1335.33	1383.00	1: 2.0
	15	87.28	88.20	87.74	1464.33	1343.33	1403.83	1: 2.5
	20	87.12	84.82	85.97	1411.33	1307.00	1359.16	1: 1.8
Ginger rhizome extract	5	81.73	75.49	78.61	1117.67	1020.00	1068.83	1: 0.4
	10	84.33	84.67	84.50	1230.00	1141.00	1185.50	1: 0.6
	15	85.69	82.98	84.33	1377.33	1279.33	1328.33	1: 1.5
	20	84.69	78.61	81.65	1321.33	1239.33	1280.33	1: 1.0
Garlic bulb extract	5	86.25	82.46	84.35	1212.00	1112.00	1162.00	1: 0.5
	10	90.57	87.92	89.24	1425.67	1306.67	1366.17	1: 1.9
	15	86.42	81.90	84.16	1308.67	1239.33	1274.00	1: 1.75
	20	85.37	74.11	79.74	1231.33	1231.33	1231.33	1: 0.7
CONTROL	0	50.57	52.50	51.53	800.67	840.00	820.33	
		Sem CD	Sem CD	Sem CD	Sem CD	Sem CD	Sem CD	
Botanicals		(±) (5%)	(±) (5%)	(±) (5%)	(±) (5%)	(±) (5%)	(±) (5%)	
		0.29 0.84	6.38 18.62	0.62 1.8	10.13 29.56	10.13 29.56	6.63 19.35	
Dose		0.33 0.97	7.37 21.50	0.71 2.08	11.69 34.13	11.69 34.13	7.65 22.34	
Botanicals x Dose		0.58 1.68	12.76 37.23	1.23 3.6	20.25 59.11	20.25 59.11	13.26 38.7	
Botanicals x Control		0.96 1.97	10.51 21.70	1.28 2.65	20.85 43.03	20.85 43.03	13.8 28.48	

ha⁻¹) and 10% of Garlic bulb extract (1306.67 kg ha⁻¹). Like the previous two years, pooled mean also showed maximum seed yield was noticed in 15% Neem leaf extract (1403.83 kg ha⁻¹) at par with 10% of this botanical (1383 kg ha⁻¹) and 10% of Garlic bulb extract (1366.17 kg ha⁻¹). Ginger rhizome extract@15% (1328.33 kg ha⁻¹), 20% dose of Neem leaf extract (1359.16 kg ha⁻¹) and 10% dose Garlic bulb extract (1366.17 kg ha⁻¹) showed no significant difference among themselves in increase the seed yield of mustard. Minimum seed yield was noticed in 5% Ginger rhizome extract (1068.83 kg ha⁻¹) followed by 5% Garlic bulb extract (1162 kg ha⁻¹), 5% Neem leaf extract (1165.66 kg ha⁻¹) and 10% Ginger rhizome extract (1185.50 kg ha⁻¹). The cost: benefit ratio also showed maximum in the plots treated with neem leaf extract (1:2.5) followed by 10% of this chemical (1:2.0), 10% garlic bulb extract (1:1.9) and 20% neem leaf extract (1:1.8).

The results proved that applications of botanicals could be biopesticidal and eco friendly substitute for chemical fungicides in management of *Alternaria* leaf blight of mustard. The success of Neem leaf extract and Garlic bulb extract in lowest blight severity with both leaves and pods which are similar with earlier reports of Meena *et al.*, 2008 and Rajendra and Lailu, 2006 though they reported that Garlic bulb extract was superior in reducing the *Alternaria* leaf blight of mustard. Whereas this experiment contradicts it that Neem leaf extract was best followed by Garlic bulb extract in reducing the *Alternaria* leaf blight of mustard in leaf, siliqua and also increase in yield with high cost: benefit ratio. This result also confirmed the results of Chattopadhyay *et al.* (2005) that Garlic bulb extract and Neem leaf extract both were the better choice than chemical fungicides for eco - friendly management of oil seed crop diseases. So, the results comprehensively proved that three application of Neem leaf extract or Garlic bulb extract @15% at 10 days interval could be bio-pesticidal and eco friendly substitute for chemical fungicide in management of *Alternaria* leaf blight of mustard. These experiment therefore suggest that water extractable plant extract may have an important roles in

biologically based management practices and that natural product may be incorporated with other management strategies. Further investigation was enabling the biopesticides application to be made available in user friendly bio-formulation for development of necessary delivery system. So that these plant extracts could be exploited for the management of diseases of mustard.

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