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Integrated pest management module for cumin (*Cuminum cyminum*) production under arid environment

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ABSTRACT

Wilt (*Fusarium oxysporum* f. sp. *cumini*), *Alternaria* blight (*Alternaria brunsii*) and aphids (*Myzus persicae*) were recorded as major pests in cumin. *A. alternata, A. brunsii* and *Cladosporium cladosporioides* were isolated from cumin leaves and stems, *Fusarium equiseti, F. oxysporum* and *Macrophomina phaseolina* were isolated from cumin roots. Pathogenicity tests validated the disease causal organisms and molecular characterization yielded novel gene sequences and have been assigned GenBank accession numbers and released in public domain. Whereas, powdery mildew, caused by *Erysiphe polygoni* was also observed causing minor aerial blight. Hitherto unknown aerial blight caused by *C. cladosporioides* in cumin was observed for the first time. The highest seed yield with the maximum reduction of diseases and aphid population was recorded in both the varieties RZ-19 and RZ-223 from bio-intensive IPM module comprising of soil amended with neem cake and vermicompost, seeds treatment with *Trichoderma viride* followed by one spray of Dithane M-45 mixed with Dinocap at 45 days, one spray of Imidachloprid at 55 days and two sprays of neem oil (2%) at 50 and 60 days after sowing *in prophylactic mode* eventually culminated in the maximum net benefit in both the varieties RZ-19 and RZ-223 of ₹ 13722 and 11787, respectively.

Key words: Aphids, Prophylactic, Soil amendment, *Trichoderma viride*

Cumin (*Cuminum cyminum* L.) native from the east Mediterranean to East India is an important spice crop grown in sandy loam to clay soils during the post-rainy season under irrigated conditions of arid to semi-arid environments. More than 90% of cumin of the world is produced in India. On all India basis the cumin production was 4850 thousand tonnes from 7601 thousand ha during the year 2016-17. About 98700 tonnes was exported from India during 2015-16 amounting to ₹ 1560 million (Anonymous 2018). Cumin is extensively used in the cuisines, antioxidants and therapeutics (Bettaieb *et al.* 2011, Nadeem and Riaz 2012). Major cumin cultivation areas in the state of Rajasthan are Jalore, Barmer, Nagaur, Jodhpur, Pali, Ajmer and Tonk districts.

The average productivity of cumin has been quite low in Rajasthan due to biotic and abiotic stresses. Many diseases and insect pests affect its productivity. The economically important fungal diseases of cumin are wilt/root rots caused by several species of *Fusarium, Macrophomina phaseolina*, *Alternaria* blight (Ozer and Bayraktar 2015) and powdery mildew in moderate to severe form (Ozer and Bayraktar 2015). Several species of *Fusarium* were found associated with cumin root rot (Hashem *et al.* 2010). Aphids (*Myzus persicae*) and caterpillars were reported as the major insect pests affecting productivity in cumin (Singh 2007). Samota *et al.* (2014) evaluated genotypes of cumin for resistance to aphid *M. persicae* and found genotypes RZ 223 and RZ 209 the least susceptible, whereas local variety and UC339 was found to be the most susceptible. The indiscriminate use of chemical pesticides in curative mode has resulted in rejection of cumin export consignments. Therefore technologies targeting integrated protection by preventing resistance to pesticides and to avoid varietal resistance to reduce biotic pressure needs to be addressed (Lamichhane *et al.* 2017). The rationale of the present study was to develop bio-intensive integrated pest management (IPM) module encompassing host plant resistance, cultural, biological and the minimum prophylactic use of chemicals to promote sustainable production with reduced environmental pollution.

MATERIALS AND METHODS

Field experiments: Field experiments were conducted in plant pathology experimental field at Central Arid Zone Research Institute, Jodhpur with two cumin varieties RZ-19 and RZ-223 during the *rabi* for three consecutive years 2015-16, 2016-17 and 2017-18 in three replications with 10 treatments in a randomized block design (RBD). The plot sizes were 2 × 4 m, row to row and plant to plant distances were 30 cm and 10 cm, respectively.

Treatments: Two cumin varieties, viz. RZ-19 and

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RZ-223 having tolerance to powdery mildew, *Alternaria* blight and *Fusarium* wilt diseases recommended for western Rajasthan were used with ten treatments : T_1= Soil amendment of neem cake @ 250 kg/ha; T_2=Vermicompost @ 2 tonnes/ha.; T_3= Seed dressing with biocontrol agent *Trichoderma viride* @ 4 ml/kg seed (1×10^9 CFU's); T_4= One spray of Dithane M-45 75%WP @ 2 ml/l mixed with Dinocap 48%EC @ 300 ml in 750 litres of water at 45 days after sowing; one spray of Imidachloprid 17.8SL @ 333 ml/ha at 55 days after sowing T_5: two sprays of Botanical: neem oil (2%) at 50 and 60 days after sowing; T_6: T_1+T_2; T_7: T_1+T_2 +T_3, T_8: T_1+T_2 +T_3+ T_4; T_9: T_1+T_2 +T_3+ T_4+ T_5, T_{10}: as control treatment with water spray.

Observations: Cumin seed yield (kg/ha), % disease severity (PDS) of leaf diseases and root rot/wilt (%) and aphids (numbers/plant) were recorded for each treatment in three replications. Foliar diseases were recorded on % leaf area infection on a 1-9 rating scale to calculate disease severity where 1= no infection (highly resistant) and 9 =more than 50 % infection (highly susceptible).

Molecular identification of pathogens: Fungal pathogens collected from diseased cumin plants were isolated on potato-dextrose agar (PDA) culture medium in Petri plates and incubated at 25°C. Each fungal pathogen was raised on to malt extract broth culture medium (for two weeks). The genomic DNA was extracted from approximately 100 mg of each fungal mycelia crushed in liquid nitrogen using Hi Media plant minikit following the protocols suggested by the manufacturer. The 5.8S rRNA gene was amplified using ITS-1 and ITS-4 primers using thermo cycler and sequenced by ABI prism DNA sequencer The identification of the fungal pathogens and designation of species was confirmed by DNA sequence analysis using BLAST program based on the maximum similarity with aligned reference sequence using the NCBI, USA.

Pathogenicity tests: All the seven cumin fungal pathogenic strains were subjected to pathogenicity under controlled conditions to confirm Koch's postulates. Cumin plants were raised in plastic pots at 25°C for one month. The pathogenic fungi *A. alternata, A. brunsii* and *C. cladosporioides* isolated from foliar parts were inoculated by spraying the spore suspension of 1×10^{5} onto the leaves of cumin plants under high humidity for three days. The root pathogens were screened by pouring inoculum suspension onto the surface of soil in each pot. Inoculated plants were maintained in pots at 25ºC with high humidity >85% for 2 weeks. Each fungal pathogen was re-isolated from diseased leaves and roots, cultural characteristics confirmed and Koch's postulates were proved.

RESULTS AND DISCUSSION

The DNA sequence analysis of rhibosomal DNA region resulted in identification of seven pathogenic fungal strains *Alternaria alternata, Fusarium equiseti, F. oxysporum, A. brunsii, Cladosporium cladosporioides* and *Macrophomina phaseolina* and have been assigned GenBank accession numbers MF 166764-67 and MH 507292-92. Besides this powdery mildew caused by *Erysiphe polygoni* was also observed as minor aerial blight. Effect of different treatments on % wilt/root rots and leaf disease severity in cumin for three consecutive years is presented in Table 1. The maximum reduction in the incidence of wilt and root rots and severity of *Alternaria* blight was recorded in IPM T_9 followed by T_8. Although aphid population varied significantly in T_9 and T_8 as compared to other treatments but both the varieties did not show significant differences with regards to aphid attack (Table 2).

Effective and economical management of cumin pests could be achieved due to the use of recommended tolerant varieties RZ-19 and RZ-223, and additive effects of soil

Table 1 Effect of different treatments on per cent wilt/root rots and leaf disease severity in cumin

Treatment	2015-16		2016-17		2017-18		2015-16		2016-17		2017-18	
	Wilt/root rots (%)						Alternaria blight severity (%)					
	RZ-19	RZ-223	RZ-19	RZ-223	RZ-19	RZ-223	RZ-19	RZ-223	RZ-19	RZ-223	RZ-19	RZ-223
T_1	9.0^{bc}	11.7^{ab}	11.3^{abcde}	14.3^{ab}	5.7^{abc}	6.0^{ab}	48.2^{abcde}	54.8^{ab}	48.1^{a}	50.4^{a}	40.7^{a}	44.4^{a}
T_2	5.3^{def}	7.3^{cde}	7.3^{bcdef}	9.7^{abcdef}	4.7^{abcd}	4.3^{abcd}	39.3^{f}	50.4^{abcd}	44.4^{a}	44.4^{a}	37.8^{a}	37.8^{a}
T_3	9.3^{cd}	12.0^{ab}	12.7^{abcd}	13.7^{ab}	6.3^{ab}	5.3^{abc}	40.7^{ef}	51.9^{abc}	48.1^{a}	44.4^{a}	17.0^{bc}	39.3^{a}
T_4	7.3^{cde}	9.0^{bc}	8.0^{bcdef}	13.0^{abc}	5.0^{abc}	4.7^{abcd}	23.7^{gh}	25.2^{g}	25.2^{cd}	22.2^{cde}	18.5^{bc}	23.7^{bc}
T_5	8.7^{bcd}	10.3^{abc}	8.7^{abcdef}	12.7^{abcd}	5.3^{abc}	6.7^{ab}	23.7^{gh}	22.2^{gh}	28.9^{bcd}	25.2^{cd}	22.2^{bc}	21.5^{bc}
T_6	3.3^{fg}	4.3^{efg}	6.7^{bcdef}	8.0^{bcdef}	4.0^{bcde}	5.0^{abc}	44.4^{cdef}	47.4^{bcdef}	40.7^{ab}	44.4^{a}	24.4^{bc}	25.2^{b}
T_7	2.7^{fg}	3.7^{fg}	7.0^{bcdef}	5.7^{cdef}	4.3^{abcd}	3.0^{cdef}	42.2^{def}	40.0^{ef}	42.2^{ab}	36.3^{abc}	25.2^{b}	22.2^{bc}
T_8	2.3^{fg}	3.0^{fg}	3.0^{f}	5.0^{def}	2.0^{def}	2.0^{def}	16.3^{h}	18.5^{gh}	15.6^{de}	23.7^{cde}	14.1^{cde}	16.3^{bcd}
T_9	1.3^{g}	1.7^{g}	2.3^{f}	3.7^{ef}	1.3^{ef}	1.0^{f}	5.9^{i}	5.2^{i}	8.9^{e}	20.7^{de}	5.9^{de}	5.2^{e}
T_{10}	9.7^{bc}	13.3^{a}	13.3^{abc}	16.0^{a}	6.0^{ab}	7.0^{a}	50.4^{abcd}	56.3^{a}	44.4^{a}	48.1^{a}	37.8^{a}	40.0^{a}
CV (%)	23.55		28.49		24.13		11.79		14.13		21.05	
LSD (0.05)	1.85		3.01		1.25		4.83		5.79		6.34	

V1= RZ-19; V2= RZ-223; Levels not connected by same letter are significantly different. The data are mean of three replications.

Table 2 Effect of different treatments on aphid population on cumin

Treatment	2015-16		2016-17		2017-18	
	RZ-19	RZ-223	RZ-19	RZ-223	RZ-19	RZ-223
T_1	28.7^{a}	27.0^{a}	15.7^{abcd}	12.0^{abcde}	17.3^{abc}	17.7^{ab}
T_2	28.0^{a}	26.0^{a}	19.0^{a}	15.3^{abcd}	20.3^{a}	18.3^{a}
T_3	25.7^{a}	25.0^{a}	18.0^{ab}	14.0^{abcd}	18.7^{a}	16.0^{abc}
T_4	17.7^{a}	18.3^{a}	12.3^{abcde}	10.3^{bcde}	14.0^{abcd}	11.7^{abcd}
T_5	20.7^{a}	22.0^{a}	14.7^{abcd}	13.3^{abcde}	15.0^{abc}	13.3^{abcd}
T_6	23.0^{a}	25.7^{a}	15.0^{abcd}	14.0^{abcd}	15.7^{abc}	16.7^{abc}
T_7	21.0^{a}	26.7^{a}	13.7^{abcd}	13.0^{abcde}	13.3^{abcd}	13.3^{abcd}
T_8	12.0^{a}	17.0^{a}	15.7^{abcd}	8.0^{cde}	16.3^{abc}	9.0^{bcd}
T_9	7.0^{a}	15.0^{a}	7.3^{de}	5.0^{e}	8.3^{cd}	5.7^{d}
T_{10}	31.0^{a}	29.7^{a}	18.7^{ab}	16.0^{abc}	20.7^{a}	19.0^{a}
CV (%)	38.09		23.05		21.70	
LSD (0.05)	9.87		3.62		3.78	

Levels not connected by same letter are significantly different. The data are mean of three replications.

amendments, neem oil and pesticides in prophylactic mode. Samota *et al.* (2014) reported cumin genotypes RZ-223 and RZ-209 as least susceptible and local variety UC-339 as the most susceptible to aphid (*M. persicae*). Lodha and Mawar (2014) reviewed cumin wilt management and summarized that in the absence of resistant sources against *F. oxysporum* f. sp. *cumini* to reduce inoculums below the economic threshold level, use of effective crop rotation, tolerant varieties, organic amendments and biocontrol agents in an integrated manner is the best way to manage this disease. Sharma *et al.* (2013) carried out field survey of the major cumin diseases and observed wilt (0-60%), blight (0-80%) and powdery mildew (0-54%) in moderate to severe form. We observed *Alternaria* blight (*A. brunsii*) and *Fusarium* wilt (*F. oxysporum*) as major diseases consecutively for three years causing substantial damage to cumin. While *A. alternata,* and *C. cladosporioides* isolated from cumin leaves and stem and *F. equiseti,* and *M. phaseolina* isolated from cumin roots besides powdery mildew (*E. polygoni*) were observed as minor pathogens. Pathogenicity of *A. burnsii* causing *Alternaira* blight of cumin was also confirmed by earlier researchers (Kumar 2004). We isolated *C. cladosporioides* causing aerial blight from leaves and stem of cumin, designated species on the basis of molecular characterization and confirmed pathogenicity. Although *Cladosporium cladosporioides* has been recently reported causing blossom blight in strawberry in Korea (Nam *et al.* 2015) but it is been reported for the first time from cumin.

Several species of *Fusarium* were found associated with cumin root rot (Hashem *et al.* 2010, Ramchandra and Bhatt 2012). Sharma *et al.* (2013) reported powdery mildew (*E. polygoni*) in moderate to severe form. Among insect pests we encountered aphids (*M. persicae*) as the major pest for proceeding three years which has also been reported as major cumin pest (Farooqi *et al.* 2005, Singh 2007). Soil application of vermicompost @ 2.5 t/ha + seed treatment with neem seed kernel extract (NSKE) followed by spray of NSKE resulted in the minimum incidence of *Fusarium* wilt, *Alternaria* blight and aphid with maximum seed yield (Shekhawat *et al.* 2016). Species of *Trichoderma* were found to be significantly superior in reducing the growth of the *A. alternata* (Gohel *et al.* 2005) and *A. burnsii* (Sharma and Pandey 2011) and *F. oxysporum* f. sp. cumini (Singh *et al.* 2007).

The year and treatment had highly significant effect on yield, diseases and aphid population studied due to significant differences in yield of different treatments on recommended varieties for Rajasthan (Verma *et al.* 2018). The non-significant effect of variety on aphid population is attributed to the fact that aphid is a highly polyphagous cosmopolitan species and have wide host range and therefore did not exhibited significant difference between the two varieties (Blackman and Eastop 2006). The non-significant effect of main factors for wilt/root rots due of perpetual soil-borne inoculums of the soil pathogens that survive for several years and cannot be eliminated completely (Piperkova *et al.* 2016).

The data of net returns as compared to the control treatment showed that treatments having one spray each of Dithane M-45, Dinocap and Imidacloprid (T_4), two sprays of neem oil (T_5), soil amendment of neem cake, vermicompost, seed dressing with *T. viride*, one spray each of Dithane M-45, Dinocap and imidacloprid (T_8) and soil amendment of neem cake, vermicompost, seed dressing with *Trichoderma viride*, one spray each of Dithane M-45, Dinocap and imidachloprid and two sprays of neem oil (T_9) resulted in higher net returns (Table 3). The IPM treatment T_9 wherein combination of soil amendment, seed treatment, three chemical sprays and two sprays of neem oil were applied has resulted in the maximum seed yield with the maximum net benefits in both the varieties RZ-19 and RZ-223 of ₹13722 and 11787, respectively.

The highest seed yield with the maximum reduction of diseases and aphid population was recorded in both the varieties from treatment T_9 wherein an IPM schedule of combination of treatments was evaluated in prophylactic mode. Shekhawat *et al.* (2013) reported that Mancozeb and neem formulations completely inhibited the mycelial growth of *A. burnsii* causing blight of cumin. The combined effect of *T. viride* along with neem seed kernel extract was found quite effective in reducing cumin wilt incidence caused by *F. oxysporum* f. sp. *Cumini* (Bhatnagar *et al.* 2013). Dinocap (Karathane) and Mancozeb (M-45) gave significant control of cumin blight caused by *A. brunsii* (Pipaliya and Jadeja 2008). Rathore (2004) reported first spraying of Dithane M-45 (0.2%) at 40 days after sowing then by two spraying of Dithane M-45 (0.2%) + Karathane (0.1%) + Dimethoate (0.03%) at 15 days interval were best in managing the blight, powdery mildew and aphid infestations on cumin. Imidacloprid (0.005%) resulted in 91.79 per cent reduction in aphid population (Jat *et al.* 2009). Application of neem

Table 3 Effect of IPM treatments on cumin seed yield and net benefit

Variety	Treatment	Yield (kg/ha)	Additional yield over control (kg/ha)	Cost of additional yield (`/ha)	Cost of chemicals and labour (₹/ha)	Net returns (₹/ha)	ICBR*
RZ-19	T_1	498.0^{cdef}	10.8	1935	4287	-2352	0.5
	T_2	512.6^{bcdef}	25.4	4565	4350	215	1
	T_3	490.0^{cdef}	2.8	499	195	304	2.6
	T_4	542.2^{abcd}	55	9898	2415	7483	4.1
	T_5	520.2^{bcdef}	33	5931	1230	4701	4.8
	T_6	522.8^{abcdef}	35.5	6397	8637	-2240	0.7
	T_7	536.5^{abcde}	49.3	8865	8832	33	1
	T_8	611.3^{ab}	124.1	22333	11247	11086	2
	T_9	632.8^{a}	145.6	26199	12477	13722	2.1
	T_{10}	487.2^{cdef}	0	0	0	0	0
RZ-223	T_1	429.1^{ef}	5	900	4287	-3387	0.2
	T_2	443.7^{def}	19.6	3530	4350	-820	0.8
	T_3	436.8^{def}	12.7	2293	195	2098	11.8
	T_4	482.0^{cdef}	57.9	10427	2415	8012	4.3
	T_5	452.2^{cdef}	28.1	5063	1230	3833	4.1
	T_6	453.0^{cdef}	28.9	5198	8637	-3439	0.6
	T_7	472.0^{cdef}	48	8631	8832	-201	1
	T_8	534.4^{abcdef}	110.3	19859	11247	8612	1.8
	T_9	558.9^{abc}	134.8	24264	12477	11787	1.9
	T_{10}	424.1^{f}	0	0	0	0	0
CV (%)				10.96			
LSD (P<0.05)]				62.82			

* ICBR= Incremental cost benefit ratio

based commercial formulation reduced the aphids population by 50% within 7 days of application (Verma 2018).

The prophylactic integrated pest management schedule is advantageous and a win-win situation because a protective IPM module prevents infection from occurring can also be applied if the disease is present at low levels and provide protection against secondary infections also. Higher net returns from treatments T_4, T_5, T_8 and T_9 were due to additive effects and significant reduction of pests that has eventually resulted in higher seed yields over untreated control. Whereas the treatments T_1, T_2 and T_6 wherein neem cake or vermicompost was applied alone or together were uneconomical due to higher cost of these treatments and low returns as in absence of other management practices could not provide protection to the cumin crop from all the diseases and aphids for an extended period up to crop maturity and harvest.

The highest seed yield with the maximum reduction of diseases and aphid population was recorded in both the varieties from T_9 wherein an IPM schedule of combination of treatments was evaluated in prophylactic mode. Wherein soil was amended with neem cake and vermicompost, seeds were sown after treatment with *T. viride*, one spray of Dithane M-45 mixed with Dinocap at 45 days, one spray of Imidachloprid at 55 days and two sprays of neem oil at 50 and 60 days after sowing *in prophylactic mode were applied* with the maximum net benefits in both the varieties RZ-19 and RZ-223 of ₹13722 and 11787, respectively. The maximum seed yield with the maximum net benefits in both the varieties RZ-19 and RZ-223 were due to the additive effects of soil amendments, seed treatment with biocontrol agents and chemical sprays against fungal diseases and aphids in treatment T_9. This integrated IPM module can be recommended for prophylactic IPM schedule of cumin pests as a plant protection module to maximize pod yield with the maximum economic benefits in moderately resistant and recommended varieties RZ-19 and RZ-223 for arid regions of Rajasthan.

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