Early blight of tomato caused by *Alternaria solani*, is responsible for severe yield losses in tomato. The conidia survive on soil surface and old dry lower leaves of the plant and spread when suitable climatic conditions are available. Macroclimatic study reveals that highest inoculum concentration of *Alternaria* spores appeared in May 2012 to 2013 and lowest concentration during January 2012 to 2013. High night temperature positively correlated and significantly \((P < 0.01)\) involved in conidial spore dispersal and low relative humidity (RH) displayed significant \((P < 0.05)\) but negative correlation with conidial dispersal. The objective of the study was to modify microclimatic conditions of tomato crop canopy which may hamper conidial dispersal and reduce disease severity. We evaluated effect of marigold intercropping and plastic mulching singly and in consortia on *A. solani* conidial density, tomato leaf damage and microclimatic parameters as compar to tomato alone (T). Tomato-marigold intercropping–plastic mulching treatment \((T + M + P)\) showed 35–39% reduction in disease intensity as compared to tomato alone. When intercropped with tomato, marigold served as barrier to conidial movement and plastic mulching prevented evapotranspiration and reduced the canopy RH that resulted in less germination of *A. solani* spores. Marigold intercropping and plastic mulching served successfully as physical barrier against conidial dissemination to diminish significantly the tomato foliar damage produced by *A. solani*.

**Keywords**: *Alternaria solani*, bliss independence, early blight, marigold, mulching

Early blight also known as target spot disease incited by *Alternaria solani* (Ellis and Martin) Jones and Grout, is one of the world’s most catastrophic disease in tomato crop. The causal organism is air borne, soil inhabiting and is responsible for early blight, collar rot and fruit rot of tomato (Datar and Mayee, 1981). The symptoms of the disease appears on leaves, stems, petiole, twig and fruits under favourable conditions resulting in defoliation, drying off of twigs and premature fruit drop and thus causing loss from 50 to 86 per cent in fruit yield (Mathur and Shekhawat, 1986). Under field conditions, symptoms of early blight occur on fruit, stem and foliage. Conidia, chlamydospores and mycelium of *A. solani* over winter on plant debris and in the soil. Atmospheric temperature, humidity, wind speed and conidial spore concentration are the factors most closely correlated with the occurrence of this disease. The primary inoculum produces conidia in the late summer, which are then splash or wind disperse to the lower leaves of the plant, where they germinate and infect (Rotem, 1994). Wind, rain and insects are the primary source of disseminating inoculum of the pathogen. *A. solani* has typical dry-dispersed spores; the conidia have slightly rough surfaces with dark-colored walls and are produce away from the host surface on aerial conidiophores (Fitt et al., 1989) and thereby are disperse from the host by gusts of wind, by acceleration forces as leaves shakes in the wind and by rain or overhead irrigation splash, through puff or tap mechanisms (van der Waals et
Marigold plants were planted alternatively with plant to
ates allelopathy against pathogen. In the field, tomato and
+ plastic mulch (T + M + P). Marigold was used as a
plastic mulch (T + P), tomato + marigold intercropping
intercropped with marigold (T + M), tomato along with
season of 2011 to 2012 and 2012 to 2013. The experiment

Materials and Methods

Experimental conditions. The experiment was con-
ducted under field conditions at the experimental research
farm of Agricultural research station, Banswara, located
at south of Rajasthan state, India. The susceptible cv. Ab-
hinav was used for the experiment during Rabi (winter)
estation of 2011 to 2012 and 2012 to 2013. The experiment
comprised of four treatments: tomato alone (T), tomato
intercropped with marigold (T + M), tomato along with
plastic mulch (T + P), tomato + marigold intercropping +
plastic mulch (T + M + P). Marigold was used as a
physical barrier to the conidial dispersal and it also cre-
ates allelopathy against pathogen. In the field, tomato and
marigold plants were planted alternatively with plant to
plant distance of 30 cm. Similarly, non intercropped to-
ma rows were 90 cm apart with 30 cm between plants.
Plastic mulch of 200 micron was spread on the bed and
30 days old marigold seedlings were transplanted during
second week of November, 15 days prior to transplanting
tomato seedlings. Plot size of each treatment was 4.5 × 3.0
m² with four replications. The inoculum of A. solani was
grown-up on sorghum grains and broadcasted in the field
on the day of field preparation for transplanting seedlings.
Also dry infected leaves of tomato showing symptoms
of early blight were powdered and dusted over the field
to serve as an inoculum. Fertilizer application was made
as per recommended dose. No fungicides were applied at
any stage of the crop. Weeding was done manually.

Disease assessment. Disease severity was recorded us-
ing the following scale: 0, no symptoms on leaf; 1, 1–5%
leaf area infected and covered by spot, no spot on petiole
and branches; 2, 6–20% leaf area infected and covered by
spot, some spot on petiole; 3, 21–40% leaf area infected
and covered by spot, spot also seen on petiole and branch-
es; 4, 41–70% leaf area infected and covered by spot,
spots also seen on petiole, branch, stem; and 5, > 71%
leaf area infected and covered by spot, spots also seen
on petiole, branch, stem, fruits. The percent damage was
estimated on the three leaflets each on terminal, middle
and lower leaves, according to the 0–5 scale proposed by
Mayee and Datar (1986).

Conidial density of A. solani under field condi-
tions. Conidia of A. solani were trapped on microscope
slide (7.5 × 2.0 cm²) coated with petroleum jelly fixed on
glass slides mounted on specially made iron stand having
glass slides erected in each experimental plot. Glass
slides mounted 1 m above ground level so that spores
trapping would not be impeded by plant growth. Spore
traps were designed to trap conidia deposited on either
side of sedimentation or impaction. Traps for sediment-
ing/impacting spores each had four vertical slides facing
different directions and one slide at mid way inside the
canopy level. The traps were replaced every week and the
number of trapped conidia was counted using Olympus
stereomicroscope. Spores trapped on each glass slide
was determined by counting the conidia in five transects
across the slide at 100× magnification and average popu-
lation of five slides was considered for analysis. As there
was no other crop with Alternaria infection, it was as-
sumed that the number of spores possibly collected and
correctly identified as A. solani. Spores on the sampling
slide were identified solely by morphological character-
istics. A simple correlation analysis was conducted on
weather variables and spore data.
Microclimatic variables. Canopy air temperature and relative humidity (RH) on every day basis were recorded with hand held digital hygrometer (288 CTH-HTC™, HTC Instruments, Navi Mumbai, India) from each plot and wind speed was recorded with iMets® automatic weather station (MMM Tech Support, Berlin, Germany) installed less than 5 meters away from experimental plot. A weekly average was obtained. Conidia of A. solani requires high humidity (≥ 75%) for at least 6 h to germinate. Thus we also calculated number of continuous hours per day (NCHPD) with canopy temperature and RH on various treatments including tomato only (T); tomato + marigold intercropping (T + M); tomato + plastic mulching (T + P); tomato + marigold intercropping + plastic mulch (T + M + P).

Macroclimatic variables. Meteorological data was obtained using iMets® automatic weather station installed less than 5 meters away from experimental plot. This device recorded temperature, RH and wind speed data every hour throughout the period of study. We did correlation analysis in order to establish the influence of maximum, minimum and mean temperature and mean RH and wind speed on Alternaria spore concentrations.

Atmospheric weather parameters may affect spore production directly or indirectly and may or may not stimulate fungal growth and spore production. Therefore, in the present study data was also evaluated for the correlation between spore counts documented on a given day and the main weather parameter. The significance was calculated at \( P < 0.01. \)

Characterizing the efficacy of combined cultural mechanisms to hinder conidial movement. The benefits of using combined application of marigold intercropping and plastic mulching compared with individual application of each mechanism were estimated in the experiment. When two mechanisms of disease management are applied concurrently, the resultant effect on the pathogen may be antagonistic, additive or synergistic. Antagonistic effects imply situations in which the efficacy of the combination of mechanisms is lower than the sum of the individual mechanism’s efficacies. Additive effects imply situations in which the efficacy of the combination of mechanisms is equal to the sum of the separate efficacies and synergistic effects imply situation in which the efficacy of the combination of mechanisms is greater than the sum of the separate efficacies. Expected disease control and synergism of early blight of tomato control were calculated using statistical model of Bliss independence given by Xu et al. (2011). The expected disease development (%) for combined use (\( D_{m1,m2} \)) is computed as the product of those two individual mechanisms (\( D_{m1} \) and \( D_{m2} \)) (i.e., \( D_{m1,m2} = D_{m1} \times D_{m2} \)). If we assumes, the treatment efficacy is defined as \( E_{m1,m2} = 1 - D_{m1,m2} \) then, \( 1 - E_{m1,m2} = (1 - E_{m1}) \times (1 - E_{m2}). \)

After algebraic simplification this leads to the commonly used formula \( E_{m1,m2} = E_{m1} + E_{m2} - (E_{m1} \times E_{m2}) \) where \( m1 \) means marigold intercropping; \( m2 \) means plastic mulching (Xu et al., 2011).

Synergy Factor (SF) = E(obs)/E(exp), where E(obs) = observed control efficacy by mixture; E(exp) = expected control efficacy by the mixture. When SF = 1, the interaction between two mechanisms is additive; when SF < 1, the interaction is antagonistic; and when SF > 1, the interaction is synergistic (Guetsky et al., 2001).

Statistical analysis. The extent of conidial dispersal of A. solani, governed by several microclimatic factors, which can be described as quantitative variables such as maximum and minimum temperature, RH and wind speed. Analysis of covariance (ANCOVA) is a general linear model with many factor variables (qualitative) and continuous variables (quantitative) which merges ANOVA and regression of continuous variables.

The ANCOVA model is given by

\[
Y_{ij} = \mu + \tau_i + \beta X_{ij} + \phi X^2_{ij} + \epsilon_{ij}
\]

where,

- \( Y_{ij} \) is the observation from j
th
 covariate of i
th
 treatment
- \( \mu \) is the general mean
- \( \tau_i \) is the effect of i
th
 treatment
- \( \beta \) is the overall slope
- \( \phi \) is the effect of i
th
 treatment on the slope of covariate
- \( X_{ij} \) is the observation from j
th
 covariate in the i
th
 treatment
- \( \epsilon_{ij} \) is the random error pertaining to j
th
 covariate of ith treatment; independently and identically distributed with normal distribution with zero mean and constant variance \( \sigma^2 \).

The identification of an appropriate Box-Jenkins model for particular time-series would first required to check for stationarity. The behaviour of auto correlation function (ACF) and partial ACF (PACF) can help to identify auto regressive moving average model that best describes the resulting stationary time-series. All statistical analyses were performed using SAS software ver. 9.2 (SAS Inc., Cary, NC, USA; SAS Institute, 2010).

Results

Effect of marigold intercropping and plastic mulching on early blight of tomato. In tomato–marigold intercropped treatment (T + M), percent disease index was
lowered by 16.1% in 2011 to 2012 and 15.7% in 2012 to 2013 experiment than tomato alone (T). Disease severity in tomato–plastic mulching treatment (T + P) reduced by 29.5% in 2011 to 2012 and 31.5% in 2012 to 2013 experiment than in tomato alone (T). Tomato–marigold intercropping–plastic mulching treatment (T + M + P) showed significant reduction in disease intensity of 38.9% in 2011 to 2012 and 35.4% in 2012 to 2013 as compared to tomato alone (T) (Table 1). Observed efficiency of combined effect of both the mechanisms such as marigold intercropping and plastic mulching to prevent conidial movement of *A. solani*, was found to be 38.9% but at the same time expected efficiency of this combination was little higher (40.4%) during 2011 to 2012. Thus SF calculated was little less than 1 (0.96). While in 2012 to 2013 observed efficiency of combined effect of both the mechanisms was 35.4% which was less than expected efficiency (42.1%). The SF calculated to be less than 1 (0.84). Therefore, the interaction judged to be antagonistic (Table 1). This refers that application of both the mechanisms performed well to hinder conidial movement but not to its fullest potential.

**Monthly spore concentration in the air of tomato crop canopy.** Significant monthly variation in conidial distribution was observed. Maximum concentration observed in May 2013 (167 spores) followed by May 2012 (155 spores) and April 2013 (146 spores) whereas low concentrations occurred in January, February, and March during two growing seasons (Fig. 1). The maximum conidial concentration was observed during period of high mean temperature, low RH and high wind speed. Fig. 2 shows the monthly changes in *Alternaria* spore concentration concurrent with meteorological parameters observed during the two crop cycles. During both the years similar pattern of maximum temperature reaching over 35°C in April and May was observed. The RH fluctuated during these years with values ranging from 55–80%. The wind velocity was very low in the month of January to March (2.6–3.6 km/h) which suddenly elevated to as high as 12.5 km/h in May 2012 and 10.9 km/h in 2013. This elevated the level of *Alternaria* spores, which was influenced by high temperature and wind velocity.

Correlation analysis was applied, taking into account *Alternaria* spore counts and weather parameters recorded on the same day. The weather parameters displayed high positive correlation coefficient with number of spores except with mean RH which was negatively correlated during both the years (Table 2). Table illustrates the correlation between meteorological data and the number of trapped conidial spores. There was a strong positive correlation (0.967) with minimum temperature, maximum temperature (0.891) and wind speed (0.793) at 1% significant level.

### Table 1. Percent disease index (PDI) caused by *Alternaria solani* in tomato plant

| Treatment       | 2011–2012 |          | 2012–2013 |          |
|-----------------|-----------|----------|-----------|----------|
|                 | PDI       | Observed efficiency (%) | Expected efficiency (%) | PDI       | Observed efficiency (%) | Expected efficiency (%) |
| T               | 41.7 (40.2)* | 0        |           | 44.0 (41.4)* | 0        |
| T + M           | 35.0 (36.1)† | 16.1     |           | 37.1 (37.1)† | 15.7     |
| T + P           | 29.4 (32.5)† | 29.5     |           | 30.2 (32.8)† | 31.4     |
| T + M + P       | 25.5 (29.9)† | 38.9     | 40.4      | 28.4 (31.7)† | 35.4     | 42.1     |
| CV              | 4.27      | -        | -         | 6.43      | -        | -        |
| SF              | -         | 0.96     |           | -         | -        | 0.84     |

*T*, tomato; *T + M*, tomato intercropped with marigold; *T + P*, tomato with plastic mulch; *T + M + P*, tomato intercropped with marigold in plastic mulch; CV, coefficient of variance; SF, synergy factor.

*Mean values followed by the same letter in a column are not statistically different.

†Bliss independence formula (two mechanisms): $E_{m1,m2} = E_{m1} + E_{m2} - (E_{m1} \times E_{m2})$, SF = $E_{\text{obs}}/E_{\text{exp}}$.  

![Fig. 1. Monthly mean number of conidia of *Alternaria solani* trapped in tomato crop canopy during cropping season of 2011 to 2012 and 2012 to 2013. Crop transplanted on 27 November 2011. Error bars are standard error values.](image-url)
cance level. However, RH showed significant \((P \leq 0.01)\) negative correlation \((-0.902)\) with the number of conidial spores trapped (Table 2). This inferred that with the increase in maximum and minimum temperature, wind speed and decrease in RH, there appeared significant increase in number of sore trapped in the later months of the season.

The regression analysis of spore population and each microclimatic variables produced the equation as given in the Fig. 3. Each of the variable has a corresponding \(R^2\) value. Study revealed that conidial spore population trapped under the influence of microclimatic conditions during the cropping season from the month of January to May months was better correlated with minimum temperature, maximum temperature, RH and wind speed with the \(R^2\) value of 0.982, 0.885, 0.962, and 0.758, respectively. It can be seen from the graph that the conidial spore count was less till March, but in April it was suddenly increased from 50 to 135. During that period, there was sudden decrease of RH from 75\% to 55\% and minimum temp increased from 13\(^\circ\)C to 21\(^\circ\)C and wind speed increased from 3.3 to 5.4 km/h.

**Table 2.** Correlation coefficients \((r)\) and significance levels between the number of spores and meteorological factors

| Weather parameter | 2011–2012  | 2012–2013  | Pooled     |
|-------------------|------------|------------|------------|
| Maximum temperature | 0.902*     | 0.895*     | 0.891*     |
| Minimum temperature | 0.961*     | 0.979*     | 0.967*     |
| Mean relative humidity | -0.896*    | -0.914*    | -0.902*    |
| Wind velocity     | 0.793*     | 0.818*     | 0.793*     |

*Significant at 1\% level of significance.

**Fig. 2.** Comparative effect of microclimatic variables like mean temperature, wind velocity and relative humidity on the number of conidia of *Alternaria solani* trapped during 2011 to 2012 (A) and 2012 to 2013 (B) cropping seasons. Error bars are standard error values. Tmean, mean temperature \(\left(\degree\right)\); Wind Vel, wind velocity \((\text{km/h})\); RH, relative humidity \(\left(\%\right)\).

**Fig. 3.** Relationship between number of spore trapped and microclimatic variables in tomato crop canopy. Method used to draw trend line is linear trend line method. Tmax, maximum temperature \(\left(\degree\right)\); Tmin, minimum temperature \(\left(\degree\right)\); Tmean, mean temperature \(\left(\degree\right)\); RH, relative humidity \(\left(\%\right)\); Wind Vel, wind velocity \((\text{km/h})\).

**Air conidial density of A. solani under field conditions.** In the four treatments (T, T + M, T + P, and T + M + P) the conidial density of *A. solani* in the air near the tomato canopies increased with time after tomato transplanting during two growing seasons, 2011 to 2012 and 2012 to 2013. However in both seasons the rate of increase in conidial density was significantly \((P < 0.05)\) diminished by intercropping or by mulching, particularly by the T + P and T + M + P treatments compared to T + M and control (T) alone. At the end of evaluation period (13 weeks after transplanting), the reductions in conidial density due to intercropping and mulching were 11.7–19.3\% for T + M association, 49.0–50.3\% for T + P association and 58.3–68.7\% for the T + M + P association in 2012 and 2013 (Fig. 4).
Altering Conidial Dispersal of *Alternaria solani*

Microclimatic changes due to intercropping. The T + P combination induced a small (3–4%) but significant reduction in the maximum RH of the air surrounding the canopy, so that NCHPD at RH ≥ 75% was significantly lower in the T + P treatment than in T + M treatment and control (T) during two different years. T + M + P treatment induced more reduction in the maximum RH (6.0–7.4%) of the air surrounding the crop canopy, so NCHPD at RH ≥ 75% was significantly lowest than the T + P, T + M and control (T) during both the years of experiment (Fig. 5). The T + M combination also reduced maximum RH and the time at RH ≥ 75% but to a lesser degree than T + P and T + M + P, thus revealed that plastic mulching is better than marigold intercropping. Association of tomato, plastic mulching and marigold intercropping (T + M + P) together was found best in maintaining lower RH in canopy as it arrested maximum humidity below plastic mulch. Minimum RH and canopy air temperature were little affected by plastic mulching and marigold intercropping alone. NCHPD with RH ≥ 75% was least (3.3 h in 2011–2012 and 4.2 h in 2012–2013) in tomato–plastic mulching–marigold intercropping association (T +

![Fig. 4](image-url). Weekly progress of air conidial density of *Alternaria solani* in tomato crop canopy with marigold intercropping (T + M), plastic mulching (T + P), and both (T + M + P) during cropping season of 2011 to 2012 (A) and 2012 to 2013 (B). Error bars are standard error values. T, tomato; T + M, tomato intercropped with marigold; T + P, tomato with plastic mulch; T + M + P, tomato intercropped with marigold in plastic mulch.

![Fig. 5](image-url). Maximum relative humidity (RH) and number of continuous hours per day (NCHPD) with high RH (≥ 75%) in tomato canopy with plastic mulching (T + P), marigold intercropping (T + M), and both (T + M + P) during cropping season of 2011 to 2012 and 2012 to 2013. Graph represents average mean of two years data (2011–2012 and 2012–2013). Error bars are standard error values. RHmax, maximum relative humidity; RHmin, minimum relative humidity.

![Fig. 6](image-url). Maximum and minimum temperature and number of continuous hours per day (NCHPD) with temperature from 22–28°C in tomato canopy with plastic mulching (T + P), marigold intercropping (T + M), and both (T + M + P) during cropping season of 2011 to 2012 and 2012 to 2013. Graph represents average mean of two years data (2011–2012 and 2012–2013). Error bars are standard error values. Tmax, maximum temperature (°C); Tmin, minimum temperature (°C).
Plastic mulching was found better than marigold intercropping in maintaining low RH in crop canopy (Fig. 5). But these intercultural operations of marigold intercropping and plastic mulching sparingly affected the temperature of crop canopy and the deviation in canopy temperature was no significant. There was very meagre variation in the canopy temperature with introduction of plastic mulching or marigold intercropping (Fig. 6).

However when ANCOVA was performed separately on microclimatic weather variables, it was found that minimum temperature significantly \( (P < 0.01) \) enhanced conidial spore dispersal to get maximum spore trapped irrespective of the month of the cropping season but maximum temperature during the cropping season did not significantly \( (P > 0.01) \) involve in spore dispersal. RH significantly \( (P < 0.05) \) affect conidial dispersal and enhanced spore trap during later months of the cropping season. Whilst wind speed non significantly \( (P > 0.01) \) involve in conidial dispersal but it was significant particularly during March, April, and May months of the season during which day and night temperature were high and RH was less.

It can also be seen from the mean scatter plot of analysis of variance (Fig. 7) that the spore count was increased in April and May month. So, weather conditions in these two months were favorable for the spore dispersal. The increase in spore count is higher in high minimum and maximum temperature of April and May (Fig. 7A, B). Also the spore count is higher in low RH of April and May months (Fig. 7C). The spore count is near about same in February and March months in all the four weather parameters conditions. Generally, the microclimatic

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**Fig. 7.** (A) Mean scatter plot of analysis of covariance (ANCOVA) for maximum temperature (MaxT). (B) Mean scatter plot of ANCOVA for minimum temperature (MinT). (C) Mean scatter plot of ANCOVA for relative humidity (RH). (D) Mean scatter plot of ANCOVA for wind speed (WS).
Altering Conidial Dispersal of *Alternaria solani*

Conditions effects on spore dispersal were stronger than that of month of the year. It was found that for conidial spore dispersion time effect was significantly correlated at 1% for all the experimental period except RH which was significantly correlated at 5% (Table 3). In general, the airborne conidial spores of *A. solani* were trapped numerous during summer months (April and May) than during late winter months (January and February). The results obtained in the present study indicated that the extent of spore dispersal of *A. solani* were highly dependent on the microclimatic conditions.

Table 2 illustrates the correlation between meteorological data and the number of trapped conidial spores. There was a strong positive correlation (0.967) with minimum air temperature significant at 1%. RH showed significant (*P* ≤ 0.05) negative correlation (−0.902) with the number of conidial spores trapped.

Analysis of ACF of spore count dataset, before any differentiation showed that there is an overall decreasing correlation factor. The autocorrelation functions are significant for a large number of lags up to lag 5 but non-significant from lag 6 to lag 10. The ACF before differentiation is close to zero at lag 6, then the series considered as white noise.

As the variables in our analysis were non-stationary, we took differences of each variable in subsequent analysis (Box and Jenkins, 1970). This approach modelled dynamic changes in the process much precisely than the traditional regression models.

The decay rate of ACF series is very slow. But after differentiating the original series the decay rate becomes high (Fig. 2) which makes the data series stationary. This step is intended to detrend the dataset to achieve stationarity in the mean and to make them homoscedastic (stationary in variance). This indicates that the data shows lag effect. The original monthly spore count dataset vis-a-vis weather parameters time series analyzed here are non stationary, and have a 5 months seasonality and are all differentiated at lag *d* = 1. It can be seen from the ACF and PACF plot (Fig. 8) of spore count data. After differentiation stationary time series showed a quasi normal distribution with near zero mean and constant variance.

We used PACF to confirm the existence of a seasonal pattern. PACF is an extension of ACF, where the dependence on the intermediate elements within the lag is removed. Note that the PACF plot has a significant spike only at lag 1 (Fig. 8B), meaning that all the higher order correlations are effectively explained by the lag 1 autocorrelation but no apparent correlation with remaining lags which suggests that one seasonal differentiation is adequate.

ACF plot shows that the correlation at lag 0, 1, and 2 are significant and positive while correlation at lag 3, 4, 5 are positive but non-significant. In particular, the PACF has only one significant spike while ACF has 6 (Fig. 8A). The present dataset is of only two years experimentation thus the lag effect visible in data is not close to conformity and realistic results could not be obtained with short experimental time period.

### Discussion

The study of microclimate and its correlation with spore concentration revealed that *Alternaria* spores were present in the atmosphere during the period of study but they appeared in higher concentration during the later stage of the crop. Similar observations were reported by different workers describing the level of the conidia in other locations, where the highest level appeared during the summer months (Grinn-Gofroń and Rapiejko, 2009; Hjelmroos, 1993). Careful observations on the initiation and development of early blight disease on tomato crop in the field revealed that the conidial inoculum reaches the leaf from the soil through mechanical or wind dispersal. Symptoms were first appeared on lower leaf canopy. Wind thereby dispersed conidial inoculum to upper leaves in addition to the lower ones. Percentage of infected leaves was higher on lower canopy level than on upper ones which suggested that the movement of inoculum from soil surface to leaves takes place by wind dispersal. Conidial spore concentration was very low at the beginning of the crop season but at crop maturity the spores were produced

| Source of variation | Degree of freedom | MS   | F      |
|---------------------|-------------------|------|--------|
| Month of the year   | 4                 | 981.14 | 7.17* |
| MinT                | 1                 | 1992.38 | 14.57* |
| CV                  | 14.89             |       |        |
| Month of the year   | 4                 | 5374.86 | 30.32* |
| MaxT                | 1                 | 614.06 | 3.46   |
| CV                  | 16.96             |       |        |
| Month of the year   | 4                 | 4841.88 | 29.67* |
| RH                  | 1                 | 1094.15 | 6.71** |
| CV                  | 16.27             |       |        |
| Month of the year   | 4                 | 10867.78 | 61.65* |
| WS                  | 1                 | 648.38 | 3.68   |
| CV                  | 16.91             |       |        |

ANCOVA, analysis of covariance; MinT, minimum temperature; CV, coefficient of variance; MaxT, maximum temperature; RH, relative humidity; WS, wind speed; MS, mean square.

*Significant at 1%. **Significant at 5%.
mainly on dead or drying leaves; therefore at crop maturity, major peaks of spore concentration were appeared. The formation of conidiophores and the spore content in the atmosphere are mainly influenced by changes in macro and microclimatic conditions which showed a strong positive correlation between spore concentration and canopy temperature. The spore content in the atmosphere increases when the mean, maximum and minimum temperatures increases, while RH decreases which suggests that *Alternaria* is a temperature sensitive, dry climatic fungus. *Alternaria* is a saprophytic genus with an optimal development shown to occur in the temperature ranges of 22–28°C in temperate regions (Hjelmroos, 1993). Wet leaves surface after dew formation and high RH along with lower wind speed reduces conidial spore dispersion but conversely high spore catches reflect dry leaves, low RH and high wind speed (Chen et al., 2003). In our study which was conducted in tropical climatic conditions, it was found that the most conidia appeared in the atmosphere when minimum temperature was 20–25°C, maximum temperature was near 40°C and mean temperature was 29–35°C during two growing seasons. It has been suggested that temperature and RH play a major role in the dispersion of *Alternaria* spores (Burch and Levetin, 2002; Marchisio and Airaudi, 2001; Stennett and Beggs, 2004). We observed that temperature was positively correlated and RH was negatively correlated with the spore population. A negative correlation was also observed with rainfall and humidity by Sabariego et al. (2000).

At the microclimatic conditions in the field, the marigold intercropping and plastic mulching in tomato crop were able to diminish significantly the tomato foliar damage produced by *A. solani*. Thus, both marigold intercropping and plastic mulching served successfully as physical barrier against conidia dissemination as reported previously by Gómez-Rodríguez et al. (2003) and Jambhulkar

Fig. 8. Cross correlation (auto correlation function [ACF] and partial ACF [PACF]) of micorclimatic variable with spore trapped before (A, B) and after (C, D) differentiation of dataset with 95% confidence band as blue shaded area. Spikes not exceeding the 95% confidence band (blue area) are no significant.
et al. (2012). In addition, it can be inferred that intercropping induced microclimatic changes in the canopy which partially prevented A. solani development. Many workers have reported that the plant protection observed in intercropping is due to changes in microclimate through differential shading (Thurston, 1992). Potts (1990) also showed a reduced severity of attack by A. solani in potato when intercropped with maize, and in tomato intercropped with maize, either in single or double rows. Kumar and Sugha (2000) registered less leaf damage caused by Stemphylium lycopersici. In the present study we reported that marigold intercropping and plastic mulching reduced the maximum RH within the canopy as well as to some extent canopy temperature presumably due to higher leaf transpiration in tomato. These microclimatic changes created additive effect by intercropping with marigold because of its antimicrobial allelopathic properties (Gómez-Rodríguez et al., 2003). That is, intercropping with marigold helped tomato plants in three different ways: one by the allelopathic effect on A. solani development; the second one by reducing maximal RH to levels lower than 75%, since this pathogen requires high humidity (Potts, 1990; Santos et al., 2000) for at least six continuous hours for fungal germination and conidia development (Pelletier and Fry, 1989; Stevenson and Pennypacker, 1988). In our study the association of tomato-plastic mulching-marigold intercropping maintained NCHPD with canopy RH ≥ 75% to 3.3 h in 2011 to 2012 and 4.2 h in 2012 to 2013. The third way, it acts as a physical barrier against spore dispersion. Plastic mulching was a barrier in the soil surface and did not allow to raise the RH of the crop canopy and thereby arresting soil moisture kept the plant turgid, vigorous and disease free. Both these intercultural operations together modified the microclimate and played a significant role in harvesting more yield as compare with monocropping and thereby give more economic return.

Use of combination of intercultural operations may cause both direct and indirect interactions among two mechanisms which may lead to increased, reduced or similar efficacy of disease management in various crops (Xu et al., 2011). However, in many such studies previously, the hypothesis of synergistic, additive or antagonistic effect of multiple mechanism of disease control was not clearly formulated and thus, not tested statistically. On application of Bliss independence, a statistical method, described above, showed antagonistic response of combined application of marigold intercropping and plastic mulching on conidial movement of A. solani during both the years of experiment. This does not implies that combined application of two intercultural mechanism is less efficient than individual intercultural mechanism. This statistical expression of antagonism suggests that either of the mechanism is performing inefficiently thus contributing less to the combined application of two mechanisms to hinder conidial dispersal of A. solani in tomato crop canopy. Use of several mechanisms concurrently for control of disease spreading inoculum fits well in the concept of integrated pest management. To best of our knowledge, this is the first report that correlates synergism of intercultural mechanism of disease control statistically using Bliss independence method. This method provides theoretical explanation of field study of reduced disease incidence by combining two intercultural mechanisms of marigold intercropping and plastic mulching to hinder conidial dispersal in tomato crop canopy. This is a novel approach to cultural control of early blight disease in tomato that may facilitate the more efficient use of this type of control on a larger commercial scale.

It is widely known and understood that ANCOVA is based on the assumptions that the relationship between dependent variables and the covariate is linear and that the slope of the line relating the dependent variable to the covariate does not differ across the different conditions in the experiment. For statistical analysis a parametric method of analysis i.e., ANCOVA without any pre-assumptions was applied. Results obtained through ANCOVA inferred the effect of individual microclimatic variables on conidial spore dispersal and verified statistically the significance of each climatic factor. In the present study correlation with microclimatic variables and number of spore dispersed was assessed quantitatively and found correlation between conidial dispersal and conditions favourable for high sporulation (usually more number of continuous hours with high humidity and high temperature). High night temperature contributes maximum in spore dispersal as low night temperature inhibit spore germination and reduced subsequent spore dispersal (Pearson and Hall, 1975). Spore trapped in early part of the crop season season reflects the non suitability of microclimatic conditions for conidial dispersal occasioned by low temperature, wet leaf surface after dew formation, high RH and lower wind speed. Conversely, high spore catches during later part of the cropping season reflect high temperature, low RH and high wind speed. Many reports suggested that other Alternaria species, significant number of spores were also dispersed by wind following drying lesions during high day and night temperature (Chen et al., 2003). The scatter plot displayed straight-line relationship between A. solani spore trapped and microclimatic variables. This may be because more number conidia tends to disperse at higher temperature and wind speed and low RH and vice versa.
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