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Full Length Research Paper

Diversity of aphids in the central rift valley of Ethiopia and their potential as vectors for Ethiopian Pepper Mottle Virus (EPMV)

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Pepper mottling is an endemic and important disease of pepper in Ethiopia. It is caused by non-persistently aphid transmitted Ethiopian Pepper Mottle Virus (EPMV) (*Potyvirus*, *Potyviridae*) . The disease occurs at epidemic level in the region. The exact roles of aphid vectors in the field epidemiology of the virus are not known. Identifying the roles of aphid vectors is useful in forecasting the epidemics of the disease. Therefore, the present studies were conducted to identify species composition and potential of aphids as vectors of EPMV. Aphids were trapped in yellow pan traps, at weekly intervals from April 2006 to March 2007 at two different locations. More than 10 aphid species were identified, of which *Myzus persicae* and *Lipaphis erysimi* were consistently dominant across locations and seasons. *Schoutedenia ramulensis* and *Tetraneura* spp. were location specific, while
Hayhurstia atriplicis, Hypermzus lactucae, Schoutedenia ramulensis and _Tetraneura_ spp. are reported for the first time from Ethiopia. Most of the identified species were non-colonizers and seven aphid species collected from field were able to transmit EPMV. The information generated has implications for the development of EPMV disease management strategies.

Key words: Aphids, Ethiopian Pepper Mottle Virus, pepper, vector

INTRODUCTION

Pepper (*Capsicum spp*.) is an important spice and vege-table crop in Ethiopia. The central rift valley is the main area of pepper production. However, the production of pepper in Ethiopia has been generally low over the last several years mainly due to severe viral infections (FAO, 2002). More than 90% viral disease incidences and com-plete crop failure have been reported from some places in Ethiopia (Agranovsky, 1993; Tameru et al., 2003).

Relative importance of viruses on pepper is quite variable across regions, where some viruses are endemic to a particular region. Ethiopian Pepper Mottle Virus (EPMV), a potyvirus occurring in mixed or single infec-tion, is the most important virus in the rift valley and sou-thern parts of Ethiopia (Agranovsky, 1993; Yaynu et al., 1999; Tameru, et al., 2003). Local and commonly grown varieties such as ‘Markofana’ are highly susceptible to EPMV and aphids play an important role as vector to spread this disease (Tameru, 2004).

The epidemiology of each virus vary with localities and time and is a factor of local source of inoculum, vector complex involved and how the pretences of vectors synchronize with the phenology of the crop (Difonzo et al., 1997). Therefore, understanding of the epidemiology of aphid-borne viruses is very important for the develop-ment of appropriate management strategies. Association between PVY incidence in potato grown in the Red River Valley of Minnesota and North Dakota and eight ineffi-cient aphid vector species has earlier been reported by DiFonzo et al. (1997).

Although, transmission of non-persistent viruses by aphids has very little specificity with respect to individual aphid species and some aphid species are more efficient in transmitting certain virus species or strains than other aphid species (Eastop, 1977). Sometimes a less efficient vector may be more important in spreading a virus if it occurs in greater abundance than more efficient vectors (Raccah, 1983). Virus incidence in soybean and potato is significantly correlated with high numbers of inefficient vectors when

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numbers of efficient vectors were low (Halbert et al., 1981; Shultz et al., 1985; DiFonzo et al., 1997).

Preliminary investigations conducted under laboratory condition revealed a non-persistent efficient transmission of EPMV by green peach aphid, *Myzus persicae* (Yaynu et al., 1999; Tameru, 2004). The capacity of aphids as vector of EPMV transmission is commonly seen in pepper and other adjacent crops. However, composition of aphid species and their role as colonizing or non-colonizing vectors in spreading the virus has not yet been investigated.

MATERIALS AND METHODS

Description of the study area

The studies were conducted at two important pepper growing locations (Awassa and Ziway) in the central rift valley of Ethiopia. Awassa and Ziway are located at an altitude of 1680 and 1600 m above sea level, respectively. Both locations are characterized by dry sub humid climate. Awassa has monthly mean minimum and maximum air temperature of 10 and 28°C and rainfall of 1400 to1800 mm per year. On the other hand, Ziway has annual rainfall of 763 to 1000 mm and mean minimum and maximum temperature of 15 and 30°C, respectively.

Farmers and private investors take three crops of pepper a year, where the August to November crop is grown on rain water, while November to March and April to August crops are grown on irrigation water. In addition, farmers also grow other crops such as maize, beans, pea, cabbage, onion, tomato, wheat, teff, barley and potato around pepper fields at both locations. Several weed species were also observed in and around pepper fields.

Composition, abundance and diversity of aphid species

Disease free seedlings of pepper variety 'Markofana' were transplanted in the field on a 100 m^2 plot area during the first weeks of April, August and December 2006. Yellow water traps (10 x 35 x 20 cm) made from yellow plastic container (Blackman and Eastop, 2000) were installed in the experimental plots. The traps were filled with water up to small outlets below the rims, and 5 ml of formaldehyde (10%) were added per trap to avoid water drinking and eating of aphids by birds from the traps.

To prevent the trapped insects from draining out during heavy rains, the outlets were covered with plastic gauze. Trapped insects were collected regularly at seven days interval. On the same day of aphid collection from yellow water trapped, non-flying aphids were also collected from ten randomly selected pepper plants using destructive sampling (Simon et al., 2009). The aphids collected from both the methods were counted separately and stored in 70% ethanol for identification purpose.

The grouping of aphids collected from field at Awassa and Ziway was done morphologically using stereomicroscope (250x) in the crop protection laboratory of Hawassa University (Blackmana and Eastop, 2000), and for further identification and confirmation, the samples were sent to the Universities of Lund and Upsala, Sweden. Specific diversity of aphids was determined by Simpson diversity index (Magurran, 1988) using the following equation:

$$D = 1 - ?^{s} (P_i)^2 \ ; \quad P_i = n_i/N$$

Where:

D = species diversity.
N = total number of individuals.

P_i = proportion of sample that contributes to the total population.

n_i = Number of individuals of i^{th} species.

The value of Simpson diversity index (1-D) ranges between 0 and 1. The greater the value of the diversity index, greater is the richness and abundance of the species (Magurran, 1988).

The potential of different aphid as Vector of EPMV

Seven most abundant aphid species were used in this experiment. These aphid species was reared separately on healthy pepper seedlings, maintained in net cages (1 × 1.5 m^2). Infected young pepper seedlings of same variety were used as a virus source. Two months old pepper seedlings were used as test plants, which before the experiment were checked for EPMV using Double Anti-body Sandwiched - Enzyme Linked Immuno Sorbent Assay (DAS-ELISA) (Clark and Adams, 1977). The anitsera raised against a virus isolate "EMPV-bod3", obtained from the sera bank of institutes of plant diseases, University of Bonn, Germany was used in the ELISA.

Hundred winged aphids of each species were taken from rearing cages in the empty petri-dishes and starved for 1 - 2 h. These starved aphid species were then released on the infected pepper seedlings and allowed to suck the cell sap for 5 - 10 min. Thereafter, these aphid species were transferred on to the healthy plants in separate compartments @10 aphids/plant. After 10 min the aphids were removed from the test plants and the test plants were sprayed with Endosulfan 39% EC. A total of 30 plants (10 plants per replication and repeated three times) were tested for each aphid species.

The un-inoculated plants were used as controls. The inoculated plants were observed for symptom development after seven days of inoculation and infected and un-infected plants were recorded for each aphid species. The transmission of EPMV by each aphid species was also confirmed with DAS/ELISA using top three leaves of each test plants. The numbers of infected and non-infected plants were counted and the data were analyzed for ANOVA using Minitab® 2007, Minitab Inc., and the significant differences in means were compared with LSD at P = 0.05. The diversity indices of the aphid species and their relative transmission efficiency for a given virus were calculated by following the method given by Nebreda et al. (2004).

RESULTS AND DISCUSSION

Composition, abundance and diversity of aphid species

A total of 3496 aphids from Awassa (Table 1) and 10,047 aphids from Ziway (Table 2) were captured from pepper during the experiment period between April, 2006 to March, 2007. Out of the total collected aphids 72% from Awassa and 66% from Ziway were collected during the peak flight activities (Simon et al., 2009) . Over 10 aphid species were identified from the collected samples. In this study, *M. persicae* (Shulzer), *Macrosiphum euphorbiae* (Thomas), *Rhopalosiphum maidis* (Fitch) , *Lipaphis erysimi* (Kaltenback) and *Sitobion* spp. were the most common aphid species encountered.

The green peach aphid, *M. persicae* and the mustard aphid, *L. erysimi* were the most abundantly found aphid

Table 1. Composition of aphid species from Awassa (2006 - 2007).

Aphid species	April	May	June	July	Aug.	Sep.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Mean	Composition of different species (%)
Aphis spp.	0	1	4	0	0	0	0	0	1	11	2	0	1.6	0.59
Hayhurstia atriplicis *	0	15	9	0	32	37	50	30	22	28	5	0	19.0	7.04
*Hyperomyzus lactucae**	2	18	21	8	20	51	1	20	18	18	11	0	15.6	5.81
Lipaphis erysimi	241	64	4	1	20	22	52	29	30	11	13	17	42.0	15.57
Macrosiphum euphorbiae	0	10	10	8	30	76	5	24	20	38	31	25	23.1	8.56
Myzus persicae	442	127	33	4	25	147	40	27	106	25	0	2	81.5	30.21
Rhopalosiphum maidis	8	11	9	3	57	69	9	25	24	45	18	0	23.2	8.59
*Schoutedenia ramulensis**	3	6	0	1	0	0	0	3	2	0	0	0	1.2	0.46
Sitobion spp.	0	13	9	4	65	40	13	4	19	50	0	0	18.0	6.70
Teteranura spp. *	0	0	0	0	0	0	0	0	0	0	0	0	0	0.00
Urolecon compsitae	9	14	2	5	0	0	0	7	5	0	0	0	3.5	1.30
Unidentified	35	22	35	0	30	0	54	254	54	3	3	1	40.9	15.17
Total	740	301	136	34	279	442	224	423	301	229	83	45	269.6	

*New records from Ethiopia.

species accounting 30 and 15% at Awassa and 30 and 46% at Ziway, respectively (Tables 1 and 2). *L. erysimi* is an important pest of *Brassicaceae* including broccoli, cabbage, Chinese broccoli, daikon, mustard cabbage and radish, and is a vector of 10 non-persistent viruses (Blackman and Eastop, 2000). Pepper has not yet been reported as a host of *L. erysimi*, indicating that the aphid is non-colonizer on this crop and act as an important vector of EPMV.

On the other hand, *M. persicae* is an established pest of pepper and found thorough out the world (Blackman and Eastop, 2000). It has been reported as a vector of EPMV from laboratory studies (Tameru, 2004) and is a well known vector of several other persistently and non-persistently transmitted viruses.

Among the identified aphid species, *Hayhurstia atriplicis* (L..), *Hyperomyzus lactucae (L.), Schoutedenia ramulensis* Rübsaamen and *Tetraneura* spp. were not reported so far from Ethiopia. *Aphis gossypii* Glover, *M. persicae* and *M. euphorbiae* have also been reported earlier from Ethiopia on pepper (Tsedeke, 1988). From the identified aphid species, *S. ramulensis* and *Tetraneura* spp. occurred less frequently than the other species in the collected samples (Tables 1 and 2). *S. ramulensis* was recorded only from Awassa, while *Tetraneura* spp. from Ziway. *Tetraneura* spp. is the gall forming aphid species (Blackman and Eastop, 2000). Some unidentified aphid species were also present in the collected samples.

Spread of viruses transmitted in a non-persistent manner by aphids often occurs when non-colonizing species land on a large number on crops (Raccah et al., 1985). It has also reported that the spread of such virus is more likely when vectors are less adapted to the virus host plant (Heatchote and Coackbain, 1964). The non-colonizer aphids were found flying to the pepper fields as soon as the pepper crop was transplanted in the field both at Awassa and Ziway (Figures 1 and 2). The high incidence of EPMV in the field reflects the abundance of non-colonizing aphids.

As pepper is not a primary host of most of the non-colonizing aphid species captured during our studies and these species might have migrated into pepper fields from other hosts including weeds. The explanation is supported by the fact that different aphid species specialize on different host species. For instance, *Hadena Atriplicis* commonly called *Chenopodium* aphid, where as *R. maidis* specializes on grasses such as sorghum and maize (Blackman and Eastop, 2000).

There is diverse cropping system at both the study locations, where maize, beans, pea, cabbage, onion, tomato, wheat, teff, barley and potato were the major crops found around the pepper fields. Several weed species such as *Amaranthus spp*, *Chenopodium* spp, *Datura stramonium, Nicandra physaloides, Solanum incanu* and *Sonchus oleraceus* were also observed in and around the pepper fields.

Similarly, the spread of Cucumber Mosaic Virus (CMV) in Alfalfa was highly associated with dispersal of different aphids from nearby Snap Beans (Nault et al., 2004). The diversity index recorded during the present studies was higher at Awassa (0.90) than at Ziway (0.75), which might be due to the large diversity of crop species cultivated at Awassa than Ziway.

The potential of different aphid as vector of EPMV

Although, there was a variation in the degree of vector competency among different aphid species, the differences in transmission rate of EPMV by test aphid species were non significant at = 0.05. The DAS- ELISA

Table 2. Composition of aphid species in Ziway (2006-2007).

Aphid species	April	May	June	July	Aug.	Sep.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Mean	Composition of different species (%)
Aphis spp.	52	6	3	4	2	2	2	0	0	0	5	0	6	0.76
Hayhurstia atriplicis	7	94	19	16	48	198	46	41	0	17	79	28	49	5.90
Hyperomyzus lactucae	0	16	14	7	0	14	22	14	16	11	0	0	10	1.13
Lipaphis erysimi	1247	445	4	0	721	112	16	250	880	997	4	2	390	46.56
Macrosiphum euphorbiae	12	37	20	9	71	176	15	33	6	5	14	21	35	4.17
Myzus persicae	701	41	25	512	712	717	37	241	3	0	9	5	250	29.89
Rhopalosiphum maidis	5	11	638	15	21	45	18	9	6	6	0	0	65	7.70
Schoutedenia ramulensis	0	0	0	0	0	0	0	0	0	0	0	0	0	0.00
Sitobion spp.	1	12	39	7	4	8	20	7	22	1	0	3	10	1.23
Teteranura spp.	15	2	0	3	0	4	49	81	1	4	2	0	13	1.60
Urolecon compositae	0	0	0	0	0	9	14	8	0	0	14	0	4	0.45
Unidentified	0	0	25	0	30	5	0	0	0	0	0	0	5	0.60
Total	2040	664	787	573	1609	1290	239	684	934	1041	127	59	837	

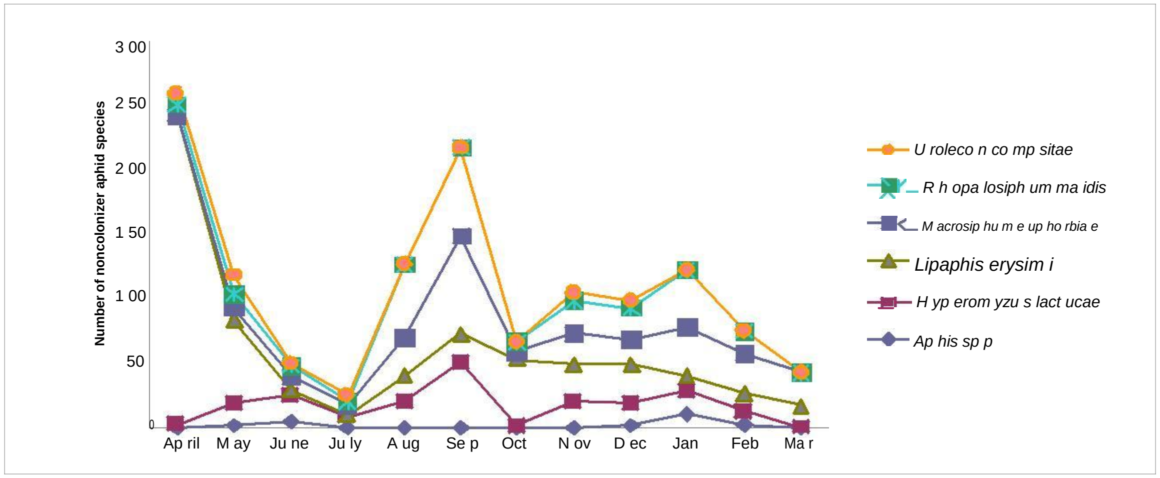

Figure 1. Population dynamics of some non-colonizer aphid species at Awassa.

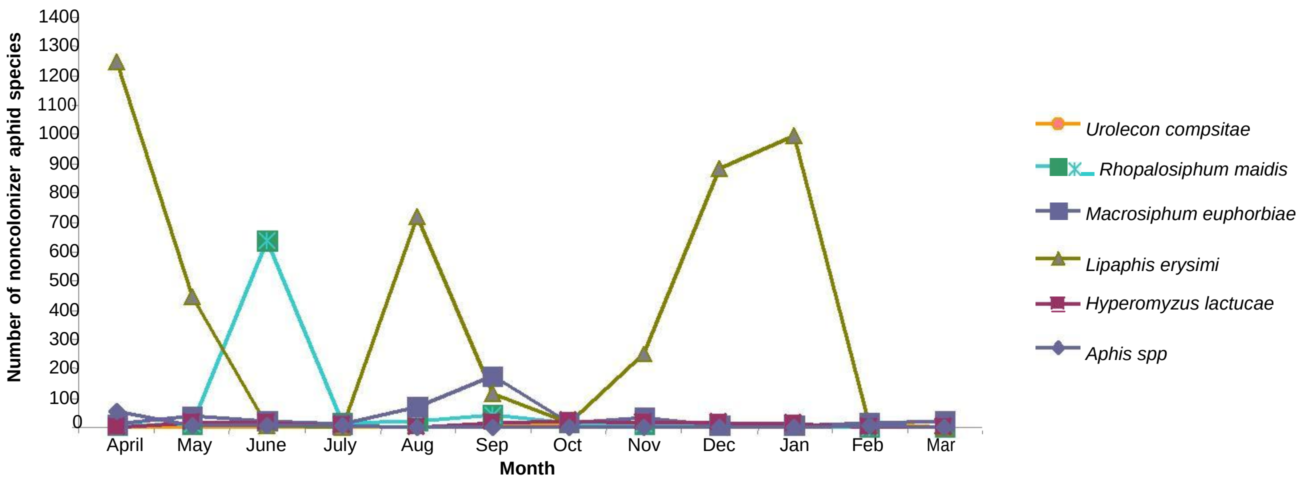

Figure 2. Population dynamics of some non-colonizer aphid species at Ziway.

Table 3. Vector competence of field collected aphid species in transmitting EPMV.

Treatment	Mean ± SE *
Control	0.0 ± 0.00a
Aphis gossypii	7.3 ± 0.74b
Hyperomyzus lactucae	8.1 ± 0.69b
Lipaphis erysimi	10.0 ± 0.62b
Macrosiphum euphorbiae	10.0 ± 0.62b
Myzus persicae	10.0 ± 0.62b
Rhopalosiphum padi	7.2 ± 0.74b
Uroleucon compositae	8.2 ± 0.69b

*10 plants were tested in three independent experiments and positive results were confirmed by DAS-ELISA. The figures following same letters in a column are statistically nonsignificant at P = 0.05.

and bioassays of pepper plants with aphids *A. gossypii*, *Myzus persicae*, *L. erysimi, M. euphorbiae*, *H. lactucae* and *U. compositae* indicated that these aphid species transmitted EPMV successfully in the pepper plants (Table 3). The *M. euphorbiae* and *M. persicae* have also been reported as vectors of Soybean Mosaic Virus (SMV) (Suzan et al., 2003), and *A. fabae* Scopoli and *A. gossypii* transmit Beet Mild Yellowing Virus (BMYV) on beet and Turnip Yellows Virus (TuYV) in oilseed rapes (Schliephake et al., 2000).

The *M. persicae* and *U. ambrosiae* also transmits non-persistent viruses such as Cassia yellow spot virus (CASV) (Souto, 1990), while Zucchini Yellow Mosaic Virus (ZYMV) is transmitted by *H. lactucae* (Katis et al., 2006). Therefore, most of the aphid species under study have been reported to transmit different persistently and non-persistently transmitted plant viruses in different crops in different parts of the world and now also been found to transmit EPMV in pepper in Ethiopia.

Conclusion

Different types of aphid species identified from pepper fields of Awassa and Ziway. Among the identified species, *H. atriplicis, H. lactucae, S. ramulensis and Tetraneura* spp., are new records from Ethiopia. *S. ramulensis* and *Tetraneura* spp. are location specific occurring at Awassa and Ziway, respectively. Most trapped aphid species are non-colonizers on pepper migrated from nearby crops and weeds.

As EPMV is non-persistently transmitted virus by several of these aphid species play a significant role in field spread of the virus. Large numbers of aphids were trapped pepper fields starting from the second week of transplanting suggesting that aphids enter pepper field at early crop growth stage. The coincidence of strong early flight activities of both non-colonizer and colonizer aphids such as *L. erysimi* and *M. persicae* and early growth stage of pepper result in severe epidemics of EPMV.

Dominant aphid species such as *Aphis* spp., *H. atriplicis, L. erysimi, M. euphorbiae, M. persicae, R. padi* and *U. compositae* were able to transmit EPMV in pepper plants under glasshouse conditions with some differential transmission ability among test aphid species. However, there is a need to generate more information on the EPMV transmission efficiency of different aphid species under field conditions to identify the most important aphid species vectoring EPMV and forecasting and management of EPMV disease epidemics.

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